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Title: Startup and Performance of a Gas-Permeable-Membrane-Supported (GPMS) Biofilm Using a Mixed Culture of Methylotrophs to Degrade Methylene Chloride, Chloroform, and Carbon Tetrachloride.

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Sandra L. Woods

The methane monooxygenase (MMO) enzyme, used by methylotrophs to oxidize methane, has been shown to possess quite broad substrate specificity, being able to oxidize many organic compounds including n-alkanes, n-alkenes, ethers, and aromatic, alicyclic, and heterocyclic compounds. In this research, it was sought to determine whether a gas-permeable-membrane-supported (GPMS) biofilm containing methylotrophic bacteria could be established and, if so, whether the methylotrophic GPMS biofilm system could be effective in degrading some recalcitrant organic compounds often found in contaminated groundwaters and industrial wastewaters.

The biofilm was grown on a gas-permeable membrane placed in a reactor vessel so as to divide the reactor into a liquid compartment and a gas compartment. Methane and oxygen were diffused from the gas compartment, through the membrane, to the methylotrophic bacteria growing on the liquid side of the membrane. Measurements

of utilization and production of various gases showed the performance of the methylotrophic GPMS biofilm to be similar to that previously observed by researchers working with methylotrophs in other types of systems. After the GPMS biofilm had reached a near-steady-state condition, a mixture of chlorinated methanes (methylene chloride, chloroform, and carbon tetrachloride) was added to the reactor liquid. Degradation and volatile losses of the compounds were monitored by gas chromatography and the methylotrophic GPMS biofilm was shown to be effective in degrading methylene chloride and chloroform. The data also suggested that the biofilm was able to supplement its electron acceptor requirements by denitrification when sufficient oxygen was not available. Enzyme kinetic constants were determined for the degradation of methylene chloride by the methylotrophic GPMS biofilm system.

Startup and Performance of a Gas-Permeable-Membrane-Supported (GPMS) Biofilm System Using a Mixed Culture of Methylotrophs to Degrade Methylene Chloride, Chloroform, and Carbon Tetrachloride

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INTRODUCTION AND OBJECTIVES

General Introduction

It has been estimated that about half of the US population uses groundwater as its drinking water source (Burmaster, 1986, Miller, 1985). While the extent of groundwater contamination is not well known, contamination by organic as well as inorganic pollutants may be widespread. For example, it has been estimated that about 3% of the nation's groundwater systems and about 4% of its surface water systems, serving as the drinking water source for an estimated 25 million persons, contain trichloroethylene (TCE) levels of 0.5 ug/L or greater, and that about 1.1 million persons using groundwater as their drinking water source are exposed to TCE levels greater than 5 ug/L (Cothern et al., 1986).

Many chlorinated organic compounds tend to be only very slowly degraded in the groundwater environment (Wilson et al., 1985; Bouwer et al., 1984). Current treatment methods generally involve removal of volatile organics by air stripping and/or removal of less volatile organics by activated carbon adsorption (Symons, 1981). These methods are relatively expensive and do not actually destroy

the organic compounds, but rather only succeed in changing their location from water to atmosphere in the case of air stripping and from water to carbon in the case of carbon adsorption. (The carbon must then either be landfilled or regenerated by incineration.) It would be very helpful if a reliable method of biological treatment could be devised to destroy these compounds by biodegradation.

Much work has been done in recent years to gain a better understanding of the methylotrophic bacteria and the methane monooxygenase (MMO) enzyme that they produce to oxidize methane. Because the MMO enzyme has been shown to exhibit quite broad substrate specificity, being able to oxidize a number of organic compounds, there has been considerable interest in determining if it would be possible to use methylotrophs to oxidize chlorinated organics often found in groundwater and industrial wastewaters. Efforts are underway to create conditions in the groundwater favorable to in situ degradation of chlorinated organics by methylotrophs (Science News, Nov 24, 1984; Science News, Sep 7, 1985). This type of approach would be advantageous in that the contaminated groundwater would not have to be pumped to the surface for treatment. Also, this approach would rely primarily on a biofilm type of growth. Biofilms tend to be very stable and effective in treating low-level concentrations of organics. One drawback that has always been associated with biofilms, however, is that after a period of time, they tend to slough.

The exact reasons for biofilm sloughing have not been well established, though it may be due to the inability of the electron

donor and/or electron acceptor to penetrate to the deepest regions of the biofilm--the portion of the biofilm attached to the support media. Without sufficient electron donors and/or electron acceptors, perhaps the bacteria attached directly to the substrate die or enter a resting stage, causing the entire biofilm to slough. If this is the case, growing the biofilm on a semi-permeable material, and providing either the electron donor or electron acceptor (or both) through the membrane directly to the attached organisms, should reduce or eliminate sloughing.

In this research, a biofilm containing a mixed culture of methylotrophic bacteria was grown on a gas-permeable membrane placed in a reactor vessel so as to divide the reactor into a liquid compartment and a gas compartment. Methane and oxygen were diffused from the gas compartment, through the membrane, to the methylotrophic biofilm growing on the liquid side of the membrane. To distinguish this type of biofilm growth from more conventional approaches, it will be referred to in this paper as a gas-permeable-membrane-supported (GPMS) biofilm. Utilization and production of various gases were monitored to assess the performance of the GPMS biofilm. After the biofilm had attained a near-steady-state condition, a mixture of chlorinated methanes was added to the liquid compartment of the reactor. The mass of each compound present in the liquid compartment and the mass of each compound lost by volatilization through the membrane were monitored to quantify degradation of the compounds by the GPMS biofilm.

Objectives

The objectives of this research were:

1. To determine the feasibility of growing a methylotrophic GPMS biofilm;

2. To identify an acceptable membrane material capable of supporting this type of biofilm;

3. To determine some of the biological performance characteristics of the GPMS biofilm, such as methane usage, oxygen usage, and carbon dioxide production;

4. To determine the kinetics and pathways of degradation of chlorinated methanes by this type of biofilm;

5. To determine losses of volatile compounds by diffusion through the membrane; and

6. To determine whether the presence of chlorinated methanes causes inhibition of normal methane metabolism.

BACKGROUND

Literature Review

Methylotrophs are bacteria that are capable of growth using reduced single carbon compounds, such as methane and methanol, as a carbon source. Obligate methylotrophs can use only single carbon compounds, while facultative methylotrophs can use other compounds in addition to single carbon compounds to support growth. Methylotrophs that have the ability to oxidize methane are known as methanotrophs. Methylotrophs are Gram-negative aerobes. Most are mesophilic. They are capable of using a number of single carbon compounds to support growth, the sole requirement seeming to be that no carbon-carbon bonds be present (Brock et al.,1984). Many are pigmented, with colors ranging from pink to yellow to ivory. Most form a resting stage during times of unfavorable growth conditions and then resume activity when conditions become more favorable (Kosaric and Zajic, 1974). Methane oxidation to methanol is accomplished by the methane monooxygenase enzyme. Methanol is further oxidized to formaldehyde, formate, and eventually to water and carbon dioxide by other constitutive enzymes.

The first documented work with methylotrophs was done in 1906 (Sohngen, 1906). He isolated a pink-pigmented, Gram-negative rod, capable of oxidizing methane, which he named Bacillus methanicus. Over about the next 50 years, relatively little information regarding methylotrophs was added to the literature. A number of researchers worked with Bacillus methanicus, especially in the

1950's and 1960's, and its name was changed several times. It is currently known as Methylomonas methanica (Kosaric and Zajic, 1974). In 1970, Whittenbury reported the isolation of more than 100 strains of Gram-negative, strictly aerobic bacteria capable of growth only on methane and methanol. He classified them into five groups on the basis of morphology, fine structure, and the type of resting stage formed. Methylotrophs are further classified as Type I or Type II, depending on whether they use the ribulose monophosphate pathway or serine pathway to assimilate carbon (Anthony, 1982).

Methylotrophs have been found to be quite ubiquitous and versatile. It seems that almost anywhere that methane and oxygen are both found to be present, methylotrophs will also be found. They have even been found at the bottom of relatively pristine, cold water lakes (Lidstrom and Somers, 1984). It is estimated that about half of the total organic carbon degraded anaerobically in nature is converted to methane, yet the amount of hydrocarbon reaching the atmosphere amounts to only about 0.5% of the total carbon turnover. Oxidation of methane by methylotrophs reportedly accounts for much of the difference between the total methane produced and that reaching the atmosphere (Higgins et al., 1980). Several types of methylotrophs have been isolated that are capable of growth on glucose and other substrates in addition to methane and/or methanol (Green and Bousfield, 1982; Lynch et al., 1980; McNerney and O'Connor, 1980; Zhao, 1984), and thermophilic methylotrophs have also been isolated (Shen et al., 1982). The ability to fix nitrogen has been demonstrated for some methylotrophs (Murrell and Dalton,

1983), as well as the ability to perform dehalogenation (Yokota et al., 1986; Brunner et al., 1980). In his work, Brunner also identified a methylotroph capable of growth using methylene chloride as its sole carbon source.

In 1959, Leadbetter and Foster reported co-oxidation of gaseous alkanes by a culture of Pseudomonas methanica (now known as Methylomonas methanica). Since that time, a great deal of work has been done to better understand the biochemistry of the methylotrophs, especially regarding the MMO enzyme and its ability to act as a biological catalyst to oxidize compounds that cannot support methylotrophic growth (see Colby and Dalton, 1976; Colby et al., 1977; Higgins et al., 1979; Hou et al., 1979a & b; Patel et al., 1979; Perry, 1979; Higgins et al., 1980; Patel et al., 1980a & b; Stirling and Dalton, 1981; Hou et al., 1981; and Patel et al.; 1982). By 1983, at least 44 compounds including n-alkanes, n-alkenes, ethers, and aromatic, alicyclic and heterocyclic compounds had been shown to be vulnerable to co-oxidation by methylotrophs (Haber et al., 1983; Burrows et al., 1984). Yokota et al. (1986) showed methylotrophic dechlorination of 1,2-dichloroethane to 2-chloroacetic acid by incorporation of molecular oxygen under aerobic conditions. He suggested that the incorporation of molecular oxygen and the inability of the organisms to dechlorinate anaerobically demonstrated the involvement of the MMO enzyme in dehalogenating haloalkanes. In a recent paper, a mixed culture of methane-oxidizing bacteria was shown to be effective in degrading

chlorinated ethenes (Fogel et al., 1986).

The possibility of the MMO enzyme to be present in either a soluble or a particulate state had been demonstrated in earlier studies (Colby et al., 1977). Burrows et al. (1984) showed the ability of the MMO enzyme in its soluble state to oxidize n-alkanes, n-alkenes, aromatic and alicyclic compounds, and the inability of the enzyme in its particulate state to oxidize aromatic or alicyclic compounds. The tendency for the enzyme to be present in one form or the other has been shown to be dependent on the copper ion concentration in the media as well as the amount of biomass present (Dalton et al., 1984). Working with Methylococcus capsulatus (Bath), Dalton found that at a copper sulphate concentration of 0.2 mg/L and a biomass concentration less than 0.8 g (dry weight)/L, the enzyme was present only in the particulate form. As the biomass concentration increased to 1.6 g (dry weight)/L, the proportion of soluble MMO increased. Above this biomass concentration, the enzyme was present only in the soluble form. In contrast, at a copper sulphate concentration of 1.2 mg/L, no soluble MMO became evident until the biomass concentration reached 1.4 g (dry weight)/L. In addition to being effective at oxidizing a more diverse range of compounds, soluble MMO has been shown to be less susceptible to inhibition than the particulate form (Dalton et al., 1984). Also, mixed cultures of methylotrophs have been shown to be less susceptible to inhibition than pure cultures (Zivotchenko et al., 1985).

In 1980, Higgins et al. suggested that due to the broad specificity of the MMO enzyme, methylotrophs play an important, and previously unrecognized, role in degrading recalcitrant compounds in the environment. Several researchers have speculated that the broad specificity of the enzyme makes methylotrophs suitable for use as biocatalysts in commercial biotransformation applications, or in water/wastewater treatment systems to treat recalcitrant compounds by co-oxidative means. Pirt (1980) suggested that methylotrophs could be useful in degrading synthetic polymers. Hamer et al. (1985) speculated that perhaps methylotrophs could be used in mixed culture with other organisms to combine degradation of recalcitrant compounds with nitrification/denitrification processes in a single treatment system. With much attention currently being focused on the problem of persistent organic chemicals in the environment, especially the groundwater environment, the possibility of harnessing the multi-faceted capabilities of the methylotrophs is a very intriguing one. Hopefully, this research will help to move us closer to solving these serious environmental challenges.

EXPERIMENTAL METHODS & PROCEDURES

System Design

Reactor

The general configuration of the reactor and its ancillaries is shown in Figure 1. The reactor was constructed of standard 4-inch inside diameter glass piping. Ports for adding and removing nutrient solution and for sampling headspace gas were installed in the liquid compartment. Ports for influent and effluent gas were installed in the gas compartment. All ports were of glass construction. A mixing rod, connected to an overhead motor and having two impellors, extended down into the liquid. The mixer rod was of glass construction while the impellors were Teflon and polypropylene. The mixing speed was 60 RPM. The lower impellor was positioned about 1-1/4 inches above the biofilm and the upper impellor was positioned just slightly below the liquid surface. The point of mixer rod penetration into the top of the reactor was sealed with silicone vacuum grease and a water seal. The reactor was built in three sections which were joined together with flanged joints. The joints were sealed with Teflon gaskets and silicone vacuum grease. The membrane was installed across the flanged joint between the gas and liquid compartments.

The reactor was located within an incubator and maintained at 30 degrees C. Temperature was measured by means of a thermometer graduated in 0.2 degree increments and secured to the outside wall of the reactor. The thermometer was insulated from the incubator

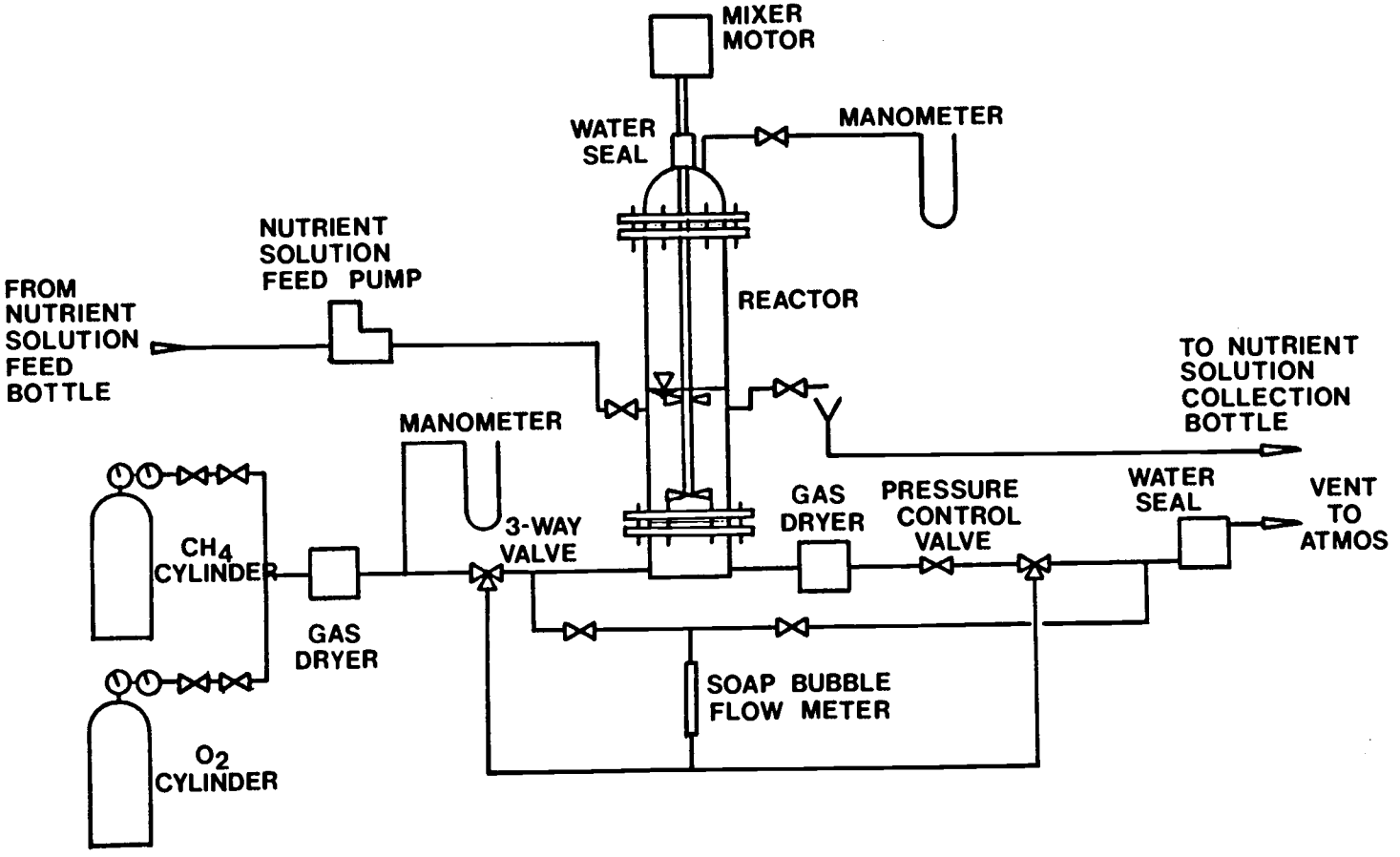

FIGURE 1 - System Schematic

air to give a more precise reading of the actual reactor temperature. Gas flow and pressure were controlled by a control valve located on the effluent side of the gas compartment. Influent gas pressure was measured by a manometer. Influent gas was dried prior to entering the gas compartment and effluent gas was dried immediately prior to entering the control valve. Calcium sulphate was used as the drying medium. Both influent and effluent gas flows to the gas compartment were measured using an in-line, soap bubble flowmeter. Upon exiting the soap bubble flowmeter the effluent gas was passed through a water seal and then vented to the atmosphere. Influent and effluent gas compositions were determined using samples collected immediately prior to and immediately after entering and leaving the gas compartment, respectively. Changes in headspace gas volume were measured by a manometer attached to the headspace nozzle. A tubing clamp was used to isolate the manometer from the headspace gas except when measurements of headspace gas volume changes were actually being made. All liquid and gas sampling ports were equipped with double rubber and/or latex septa to minimize losses of volatile compounds through the sampling ports.

The reactor was designed to operate in either batch mode or continuous-flow mode with respect to the liquid compartment, but was operated in batch mode throughout this testing. The gas compartment was operated in a continuous-flow mode. A pump was used to supply nutrient solution to the liquid compartment on an intermittent basis. Removal of nutrient solution from the liquid compartment was by gravity drainage to a collection bottle. All liquid and gas

tubing was standard 1/4-inch Tygon tubing.

Nutrient Media

Nutrient media in the liquid compartment was a variation of the nitrate mineral salts media used by Whittenbury et al. (1970). Its composition is shown in Table 1. While the distilled water contained 30 to 40 ppb copper, care was taken to eliminate all other copper from the nutrient solution.

Table 1 - Nutrient Media Composition

Ingredient	Amount Added
$MgSO_4$	488.4 mg
$CaCl_2$	101.3 mg
EDTA Disodium Salt	4.11 mg
KNO_3	1000 mg
Trace Elements Solution	0.5 ml
KH_2PO_4	272 mg
Na_2HPO_4	284.4 mg
$FeCl_3.6H_2O$	3.0 mg
$(NH_4)_2HPO_4$	1.0 mg
Distilled Water	1 L

Trace Elements Solution

EDTA Disodium Salt	500 mg
$FeSO_4.7H_2O$	200 mg
$ZnSO_4.7H_2O$	10 mg
$MnCl_2.4H_2O$	3 mg
H_3BO_3	30 mg
$CoCl_2.6H_2O$	20 mg
$CaCl_2$	0.75 mg
$Ni(NO_3)_2.6H_2O$	2.45 mg
$Na_2MoO_4.2H_2O$	3 mg
Distilled Water	1 L

Membrane Material

Several membrane materials were evaluated. The material selected for use was a Teflon/nylon laminate manufactured by W.L.Gore & Associates, Elkton, MD., and is commonly known as Goretex.

Bacterial Seed

The bacterial seed consisted of 10 ml of settled trickling filter effluent from the Corvallis, Oregon municipal wastewater treatment plant and 10 ml of settled sludge from a bench-scale anaerobic reactor in operation at the Oregon State University Environmental Engineering Laboratory. Microscopic examination of the reactor liquid a short time after seeding revealed a very diverse culture of higher and lower microorganisms. Over a period of time, the culture was enriched for methylotrophs by providing methane as the principal carbon source and by maintaining a moderate flow of nutrient solution to wash out undesired organisms. During the test period described in this paper, the reactor liquid looked very clear and the only bacterial growth visible was that actually growing on the surface of the membrane.

Approach

Methane and Oxygen Utilization

Methane and oxygen utilization were determined by performing a mass balance around the liquid and gas compartments. Flows, compositions, and pressures were measured for the gas compartment influent and effluent gas. Headspace gas volume and composition were measured to determine how much of each particular gas was passing through the membrane and water column to accumulate in the headspace at the top of the liquid compartment. Using this approach, it was also possible to determine if gases were transitioning in the opposite direction, i.e., away from the headspace and toward the biofilm. Temperature readings were taken each time gas measurements were made, and all gas measurements were corrected for temperature effects.

Carbon Dioxide Production and Nitrogen Production/Utilization

Carbon dioxide production and nitrogen production/utilization were determined in the same manner as methane and oxygen utilization.

Ability to Degrade Chlorinated Methanes

Degradation of chlorinated methanes was determined by performing a mass balance around the gas compartment and the liquid in the liquid compartment. The concentration of each compound in the liquid compartment headspace gas and liquid was measured directly to determine the mass of each compound present in each

phase. (It had been desired originally to use headspace monitoring techniques [Gossett, 1985] to determine the mass present in each phase, but it was learned that the compounds did not equilibrate between the liquid and gas phases quickly enough to allow this method to be used confidently.) The amount of each chlorinated compound diffusing through the membrane and exiting the reactor with the gas compartment effluent gas was determined by measuring the concentration of each compound in the effluent gas and the gas flow rate, temperature, and pressure. Because of the extensive precautions taken against loss of the compounds through septa, ports, etc., it was assumed that such losses were insignificant in the mass balance. As will be discussed later, the data seem to support the validity of this assumption.

Analytical Methods

Gas Compositions

Gas compositions (methane, oxygen, nitrogen, and carbon dioxide) were determined using a Fisher Model 25V gas partitioner. The gas partitioner used two columns, one packed with 30% BEEA on 60/80 Chromosorb PAW to separate carbon dioxide from the gas stream and the other packed with 45/60 Molecular Sieve 13X to separate the other gases. The packings were obtained from Supelco, Inc., Bellefonte, PA. Output data from the gas partitioner were recorded on a Series D5000 Omniscribe strip chart recorder. Measurements of gas peak heights and construction of gas standard curves were done manually. A standard curve was determined for each gas every time

gas composition measurements were made. Gas samples (100 uL) were collected using a 500 uL Pressure-Lok, gas-tight syringe and immediately injected into the gas partitioner for analysis.

Gas Flows

Because gas flows were very small (usually less than 10 ml/min), the only type of gas flow measuring device found to be reliable was a soap bubble flowmeter. Several different rotameter-type flow measuring devices were tried, but all were found to yield inconsistent and unreliable results. Also, because gas flow measuring devices are almost always calibrated for air or pure gases, the varying composition of the influent and effluent gases made calibration of these devices difficult and time consuming. By measuring the rise rate of a soap bubble inside the calibrated tube of the flowmeter, a direct reading of gas volume per unit time was obtained. The soap bubble flowmeter was permanently installed in the gas lines such as to allow direct measurement of either the influent or the effluent gas flow by changing two three-way valve settings and closing one tubing clamp while opening another (Figure 1). This method was found to be desirable because it minimized the amount of disruption to the system while providing a consistent basis for measuring the influent and effluent gas flows. Because of the small gas flows and small gas volumes in the gas compartment and gas lines, the gas system was very sensitive to disruptions of any sort.

All gas flow measurements were corrected for temperature and

pressure. A manometer connected to the influent gas line provided a continual display of the membrane inlet gas pressure. Inlet gas pressure was maintained at a level just slightly greater (< 7 inches of water column) than that necessary to counterbalance the pressure on the liquid side of the membrane. For example, if the height of the liquid above the membrane was 9 inches, the inlet gas pressure was controlled at about 9 to 16 inches of water column. The inlet gas pressure was not tightly controlled, but it was closely monitored to allow proper corrections of gas flows. Effluent gas pressure was determined by the height of the water in the final gas effluent water seal. This value was held constant throughout the testing.

Oxygen Transfer Evaluations

Oxygen transfer was evaluated using standard reaeration measurement techniques, except that the reactor was sealed to ensure that re-oxygenation of the reactor liquid was due only to oxygen diffusing through the membrane. The reactor contained one liter of distilled water for all oxygen transfer tests. The reactor contents were first deoxygenated by bubbling nitrogen into the reactor liquid with a sparger. When the oxygen concentration in the water had dropped to less than about 0.5 mg/L, the nitrogen was turned off and the reactor immediately sealed.

Oxygen was supplied continuosly to the reactor gas compartment so that it would not accumulate nitrogen. Influent oxygen pressure was kept between 5 and 10 inches of water column during the testing.

The oxygen concentration was measured with a YSI dissolved oxygen probe and YSI Model 57 D.O. meter and was continuously recorded on a Series D5000 Omniscribe strip chart recorder. Determination of $K_L a$ was by standard techniques using a plot of the natural log of the difference between the saturation D.O. concentration and the D.O. concentration at any time, t, versus time. Standard book values were used for the saturation D.O. concentrations. The maximum membrane oxygen transfer capability was calculated by correcting $K_L a$ to 30 degrees C and by assuming that maximum oxygen transfer would be attained when the oxygen concentration on the liquid side of the membrane was equal to zero.

Chlorinated Organics Concentrations

Concentrations of chlorinated compounds were determined using a Hewlett-Packard Model 5890A gas chromatograph equipped with a Hewlett-Packard Model 3392A integrator. A 1/4-inch glass column, 8 feet in length and packed with 60/80 Carbopack B with 1% SP-1000, was used in conjunction with a flame ionization detector. Nitrogen, flowing at a rate of 40 ml/min, was used as the carrier gas. Hydrogen and air flows to the hydrogen flame were 20 ml/min and 200 ml/min, respectively. Gas and liquid samples were withdrawn from the reactor with 1 mL and 500 uL Pressure-Lok gas-tight syringes, respectively, and injected directly into the GC for analysis. Injection volumes were 500 uL for gas samples and 100 uL for liquid samples. Temperature programs used on the GC are shown in Table 2. The injector port and detector temperatures were 200 and 250 degrees

C, respectively, for all analyses. Using these temperature programs, retention times for the compounds tested are shown in Table 3. Including the time required for the oven to cool down between injections, each injection required about 20 minutes of GC time.

Table 2 - Temperature Programs Used

for GC Analysis of Chlorinated Methanes

Liquid Samples:

Initial Oven Temp: 45 degrees C for 1.5 minutes

First Ramp: 20 degrees C/minute to 100 degrees C with a 2-minute hold at 100 degrees C

Second Ramp: 10 degrees C/minute to 150 degrees C with a 5-minute hold at 150 degrees C

Gas Samples:

Initial Oven Temp: 40 degrees C for 2 minutes

First Ramp: 20 degrees C/minute to 150 degrees C with no hold at 150 degrees C

Second Ramp: 10 degrees C/minute to 200 degrees C with a 4-minute hold at 200 degrees C

Table 3 - GC Retention Times of Chlorinated Methanes

	Liquid Samples	Gas Samples
Methylene Chloride	4.1 min	4.7 min
Chloroform	7.2 min	7.3 min
Carbon Tetrachloride	9.0 min	8.5 min

Attempts were made initially to employ solvent extraction techniques for the liquid samples, but unsatisfactory results were obtained. Hexane and iso-octane were both tried during these attempts. Because a purge-and-trap apparatus was not immediately available, it was decided to use direct injection of the reactor liquid. This technique worked acceptably, although it required a relatively large injection volume. The method provided consistent results throughout the testing period.

RESULTS

Startup and Membrane Evaluation

When the reactor was originally started up, it was thought that any gas-permeable membrane material would be acceptable. For about six weeks after the reactor was seeded, growth of methylotrophs did not occur on the membrane. During this time, several things were done to try to encourage growth. Other nutrient solution formulations were tried, in addition to various proportions of oxygen and methane. Still, no methylotrophs grew on the membrane. During one period when the top of the reactor was open to the atmosphere and methane was being bubbled directly into the reactor liquid using a sparger, pink growth became evident. The growth was attached to the reactor wall just slightly below the surface of the liquid and in other oxygenated areas. Because of this observation, it was suspected that the membrane was not effectively passing oxygen through to the liquid compartment. Membrane oxygen transfer evaluations were immediately undertaken. The reactor liquid was saved so that the reactor would not need to be re-seeded after the oxygen transfer tests were completed. The tests revealed that the membrane was in fact transferring only a minute amount of oxygen to the reactor liquid. Other membrane materials and slightly modified mixer configurations were tested for oxygen transfer. Test results are summarized in Table 4.

Table 4 - Results of Membrane Oxygen Transfer Tests

Membrane Material	K_La Hr^{-1}	O_2 Transfer $mg/d\text{-}cm^2$
1. Nylon/TFE* with Nylon Up (Original Reactor Configuration)	0.054	0.096
2. TFE on PP** Scrim with TFE Up (0.2 micron Nominal Pore Size)	0.522	0.926
3. TFE on Non-woven PP with TFE Up (0.2 micron Nominal Pore Size)	0.144	0.256
4. TFE on Non-woven PP with TFE Up (1.0 micron Nominal Pore Size)	0.240	0.426
5. TFE on Woven Nylon with TFE Up (Impellor 4 inches Above Membrane)	0.395	0.701
6. TFE on Woven Nylon with TFE Up (Impellor 2 inches Above Membrane)	0.794	1.41
7. TFE on Woven Nylon with TFE Up (Impellor 1-1/4 inches Above Membrane)	1.19	2.11

* TFE - Teflon

** PP - polypropylene

Based on the outcome of these tests, the configuration used in test 7 was selected. It was decided to position the membrane with the Teflon side up to take advantage of the natural hydrophobicity of the Teflon to reduce the diffusion boundary layer. It was not known whether having the Teflon side up would make any difference in oxygen transfer after biofilm growth had been established, but it did seem to enhance gas transfer significantly for the bare membrane material.

Within one week after the new membrane was installed, pink growth began to be evident on the membrane. The reactor was operating with roughly a 50:50 mixture of methane and oxygen being supplied to the gas compartment. After about three weeks, the pink methylotrophic biofilm looked to be firmly established. Unfortunately an incubator malfunction during the fourth week caused the temperature to go up to over 50 degrees C. This condition occurred late one night and was not discovered until the next morning. Some of the biofilm sloughed off over the next two or three days, apparently because of the high temperature. (As of the date of this writing, the reactor has been operating for about five months. No other incidences of sloughing have been observed.) A gas leak in the gas compartment was discovered at that time also. The reactor was completely disassembled, the leak was repaired, and all joints were sealed with silicone vacuum grease in addition to the gasket seals that had been used previously. The gas compartment was then kept immersed in a water bath to enable immediate detection of any future gas leaks. No other incidents of joint leakage were

detected throughout the course of the testing. After the reactor was reassembled and started up again, it took about two weeks for the methylotrophic biofilm to once again become thick on the membrane. At this time, about six weeks after the new membrane had been installed, routine monitoring of reactor performance began. The time line origin on all performance graphs corresponds to this point in time.

Biofilm Biomass Production

After all testing was completed, the reactor was partially disassembled and a portion of the biofilm was scraped off to determine the amount of biomass present. It was found that the total bacterial mass on the membrane was about 0.6 grams (dry weight). Since the membrane surface area was 81.1 square centimeters, this corresponded to a biofilm areal density of about 7.4 mg of biomass (dry weight) per square centimeter of membrane surface. The depth of the biofilm was also measured and was found to vary across the surface of the membrane. The biofilm was about 1 mm thick at its outer edge and increased to about 3 mm at its center. This would indicate a total biofilm volume on the membrane of about 32.4 cubic centimeters and an in situ biomass density of 18.5 mg (dry weight) per cubic centimeter of biofilm. In more conventional terms, this would be equivalent to a cell density of about 18,500 mg (dry weight) per liter of biomass volume. The total suspended solids in the reactor bulk liquid was determined to be 5 mg/L.

Methane and Oxygen Utilization

Biofilm methane usage throughout the test period is shown in Figure 2. While it was very difficult to measure the very small differences in gas composition and flow between the influent side and the effluent side, causing a fair amount of scatter in the data, a definite trend is apparent. The shaded data points were collected while the chlorinated compounds degradation test was in progress. (Very erratic results were obtained for all gas measurements during the period from day 13 to day 19. This occurred because the gas compartment water bath had been removed. Without the water bath, it was found that merely opening the incubator door to take gas flow measurements caused changes in temperature and pressure sufficient to cause gas flow measurements to vary wildly. When the source of the problem was identified, the water bath was replaced and the variation in flow measurements settled down to an acceptable level. These data are not shown on any of the figures.)

As mentioned in a previous section, about six weeks elapsed between the time the new membrane was installed and the beginning of the time period covered by the time line. According to a linear regression analysis of the data in Figure 2 (excluding erratic data during the time period described above and data during the chlorinated compounds test), methane usage increased from about 9.2 mmol/day to about 17.3 mmol/day over the test period ($r^2 = 0.36$). Based on the biomass determinations discussed in the previous section, the methane usage amounted to about 28.8 mmol of

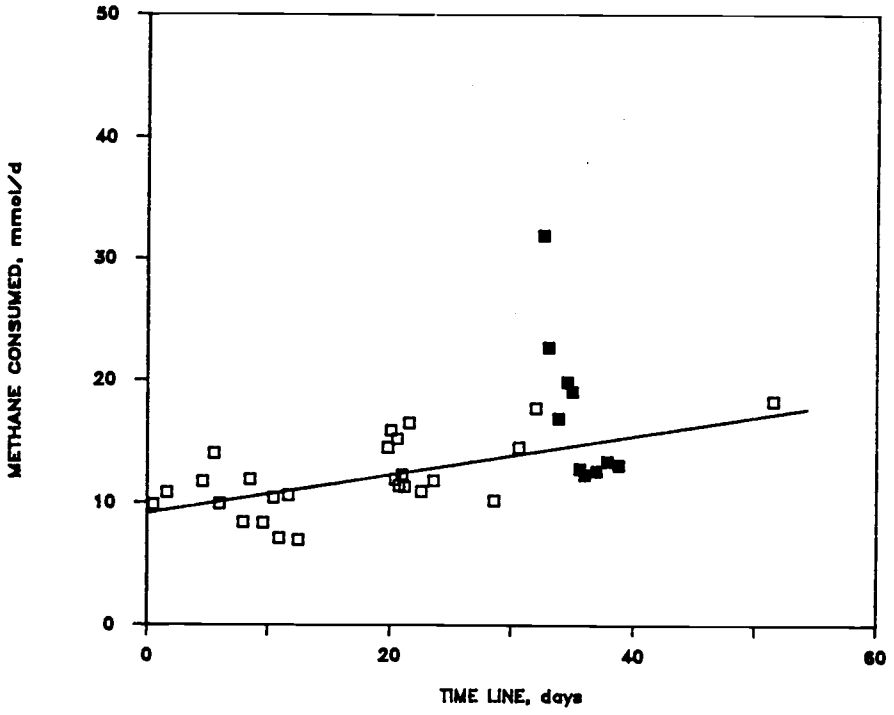

FIGURE 2 - Daily Methane Consumption

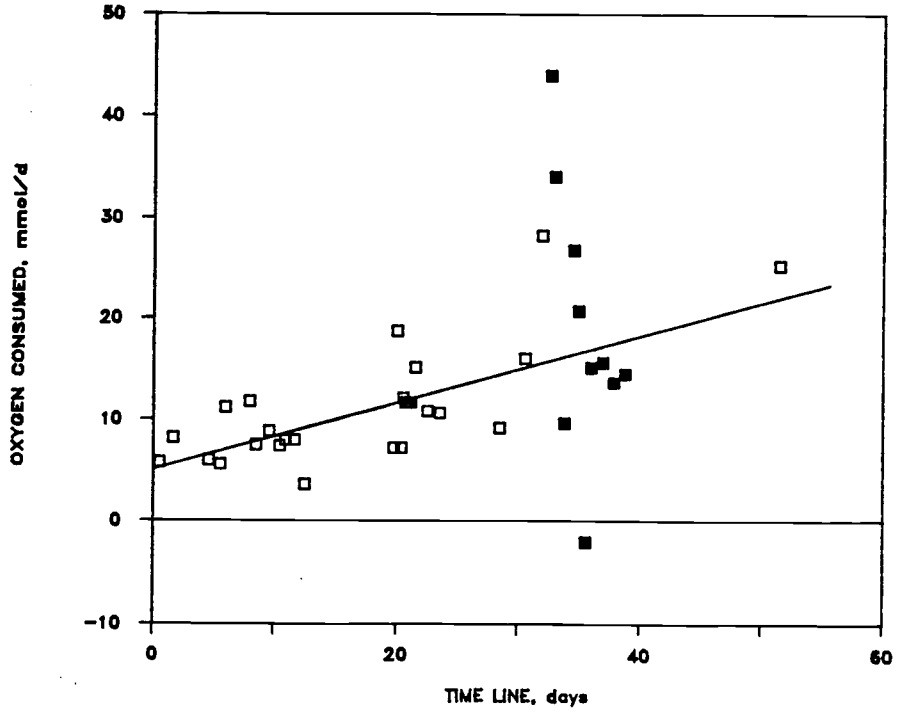

FIGURE 3 - Daily Oxygen Consumption

methane per day per gram of biomass (dry weight) at the end of the test period, or about 0.46 grams of methane per day per gram of biomass (dry weight).

Figure 3 shows oxygen consumption during the test period. Linear regression analysis (excluding the same data points as above) shows that oxygen utilization varied from about 5 mmol/day to about 21.7 mmol/day over the test period (r^2 = 0.57). This would indicate a molar ratio of oxygen:methane utilization of about 0.54:1 at the beginning and 1.25:1 at the end of the test period. Biomass oxygen consumption at the end of the test period was about 36 mmol of oxygen per day per gram of biomass (dry weight), or about 1.1 grams of oxygen per day per gram of biomass (dry weight).

Carbon Dioxide and Nitrogen Production

Production of carbon dioxide throughout the test period is shown in Figure 4. A linear regression analysis of the data indicates that carbon dioxide production increased from about 1.8 mmol per day to about 2.5 mmol per day over the test period (r^2 = 0.02). This corresponds to carbon dioxide production rates of about 0.2 mmol per mmol of methane degraded at the beginning of the test period and about 0.15 mmol per mmol of methane degraded at the end of the test period. A marked increase in carbon dioxide production is evident during the time that the chlorinated compounds were in the reactor.

Nitrogen production/consumption is shown in Figure 5. Early during the test period, about 5.8 mmol of nitrogen gas was being

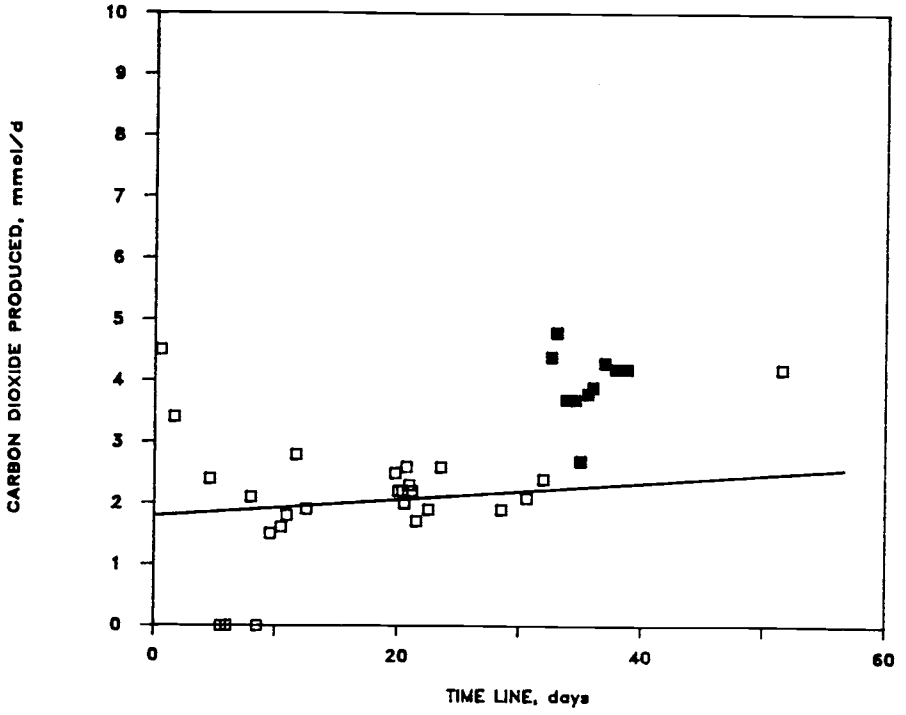

FIGURE 4 - Daily Carbon Dioxide Production

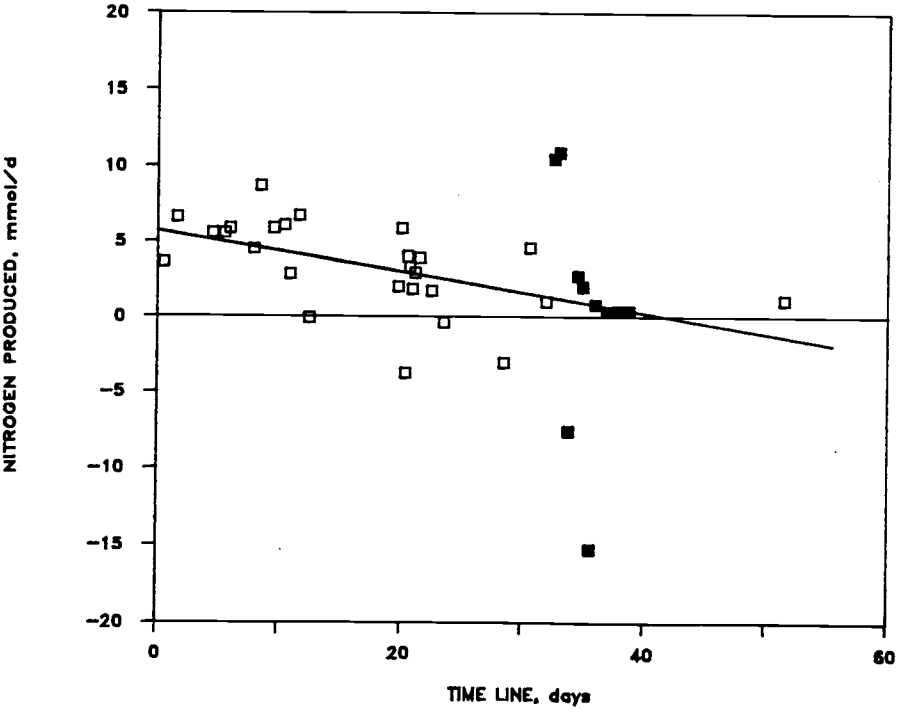

FIGURE 5 - Daily Nitrogen Production

produced per day. According to a linear regression analysis of the data, nitrogen production steadily decreased as the test progressed, until about 1.6 mmol of nitrogen gas was being consumed per day at the end of the test (r^2 = 0.27).

COD Balance

Figure 6 shows the results of a daily COD balance around the reactor. In spite of changes in methane and oxygen consumption over the test period, COD consumption remained fairly steady at about 5.5 mg of COD per day per square centimeter of membrane area (r^2 = 0.001). This amounts to about 0.74 mg of COD consumed per day per mg of biomass (dry weight), based on the amount of biomass present at the end of the test period.

Degradation of Chlorinated Compounds

Figures 7 through 15 show the concentration of each of the chlorinated compounds in the reactor liquid, the headspace over the liquid, and in the gas compartment effluent gas during the test period. For reference, the chlorinated compounds degradation test was carried out on days 32 through 39 on the time line shown on Figures 2 through 6. It is interesting to note that a significant period of time was required to attain an equilibrium distribution of the compounds between the liquid phase and the gas phase within the reactor liquid compartment. Based on the plots of concentration in the headspace versus time, approximately 12 to 16 hours was required for the methylene chloride, 20 to 24 hours for the chloroform, and

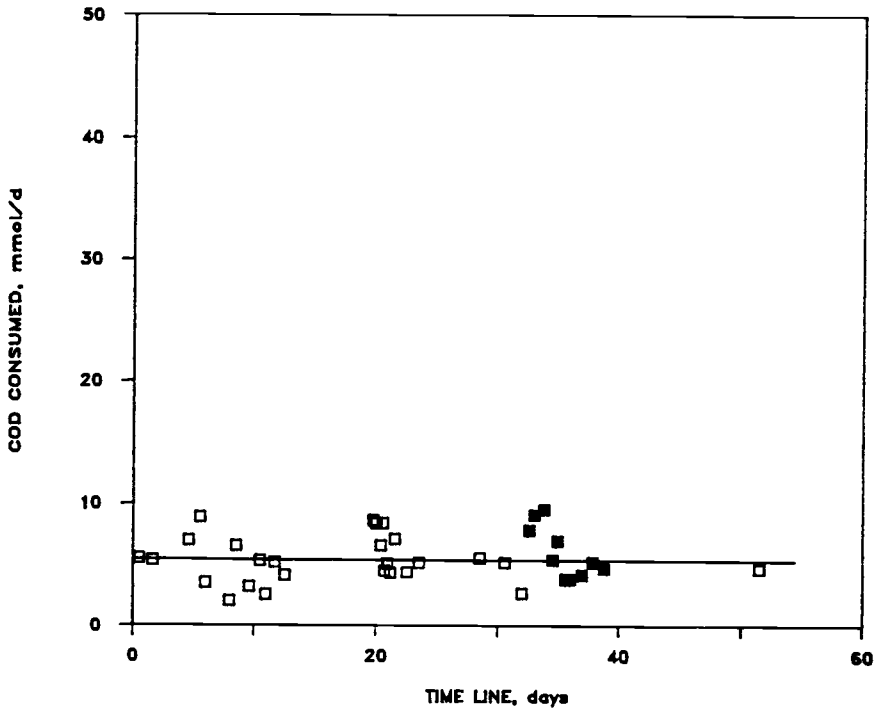

FIGURE 6 - Daily COD Consumption

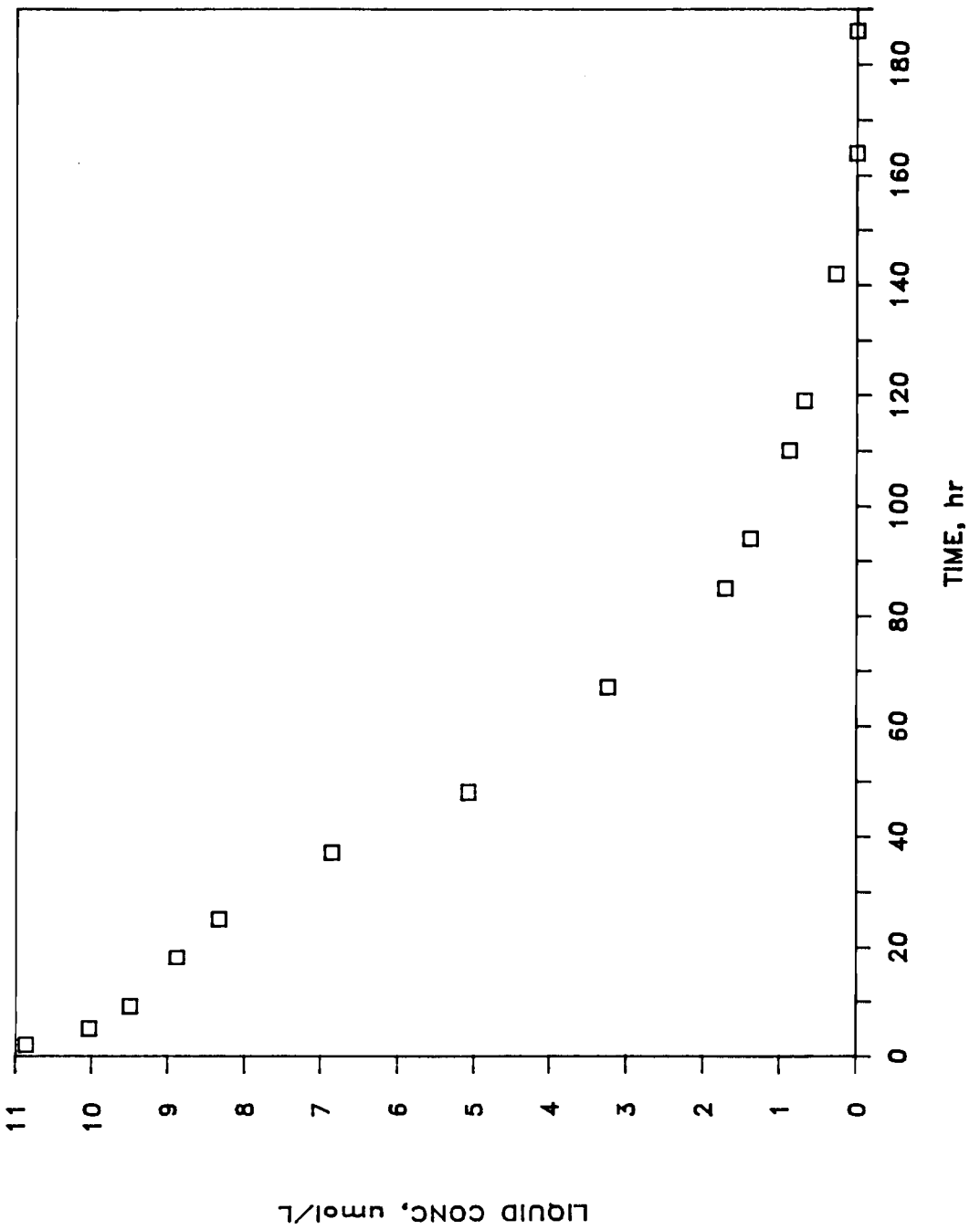

FIGURE 7 - Concentration of Methylene Chloride in Reactor Liquid

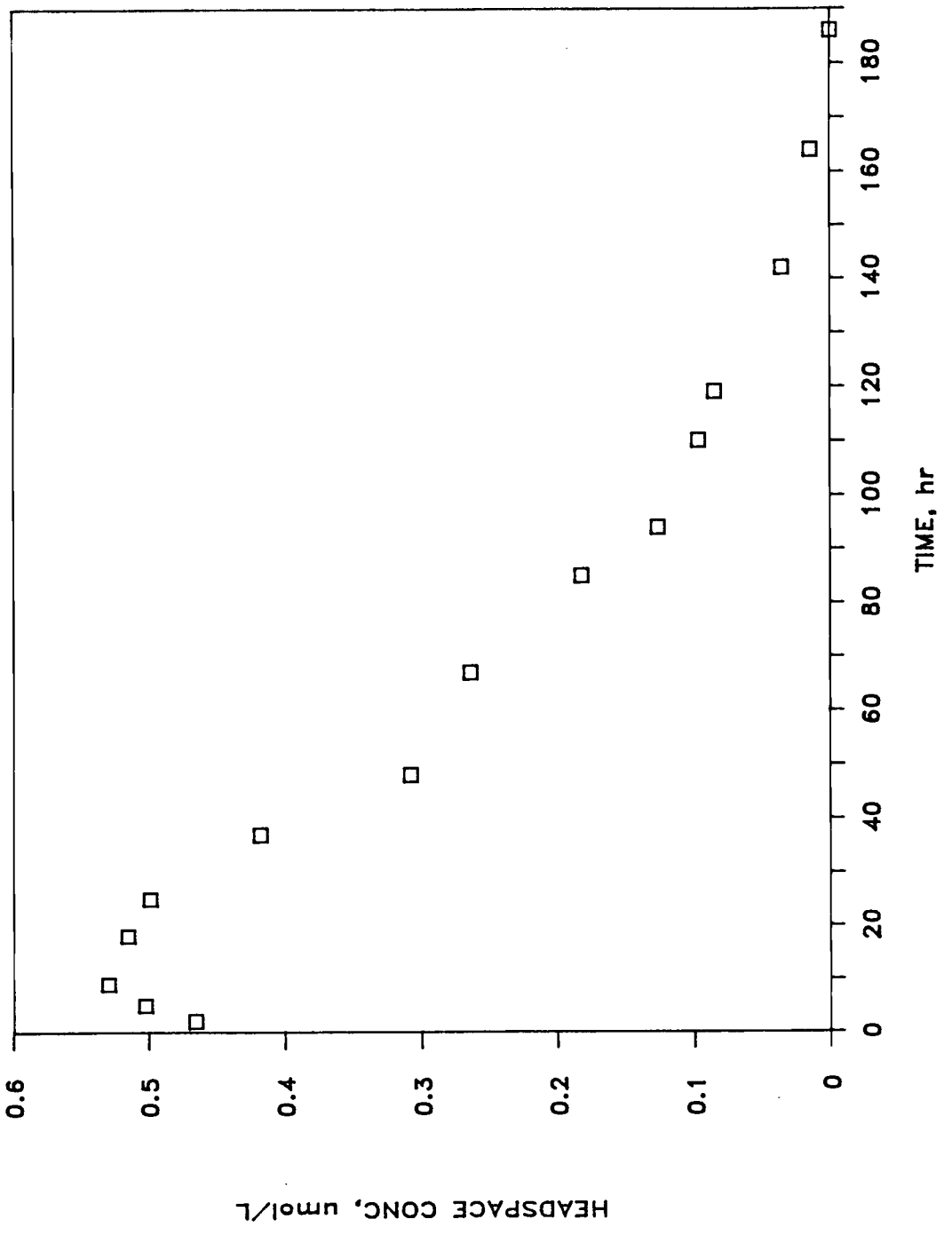

FIGURE 8 - Concentration of Methylene Chloride in Reactor Headspace

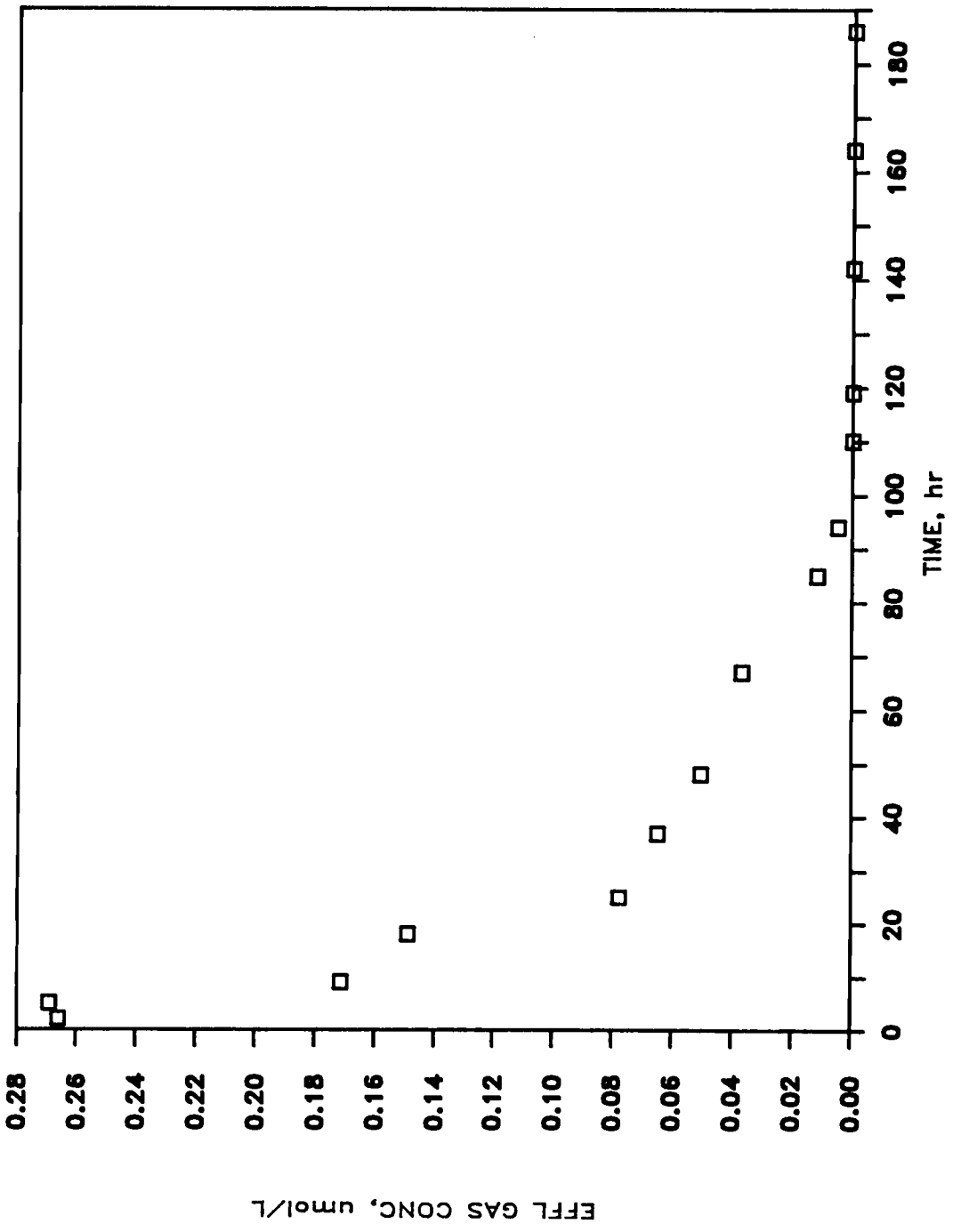

FIGURE 9 - Concentration of Methylene Chloride in Effluent Gas

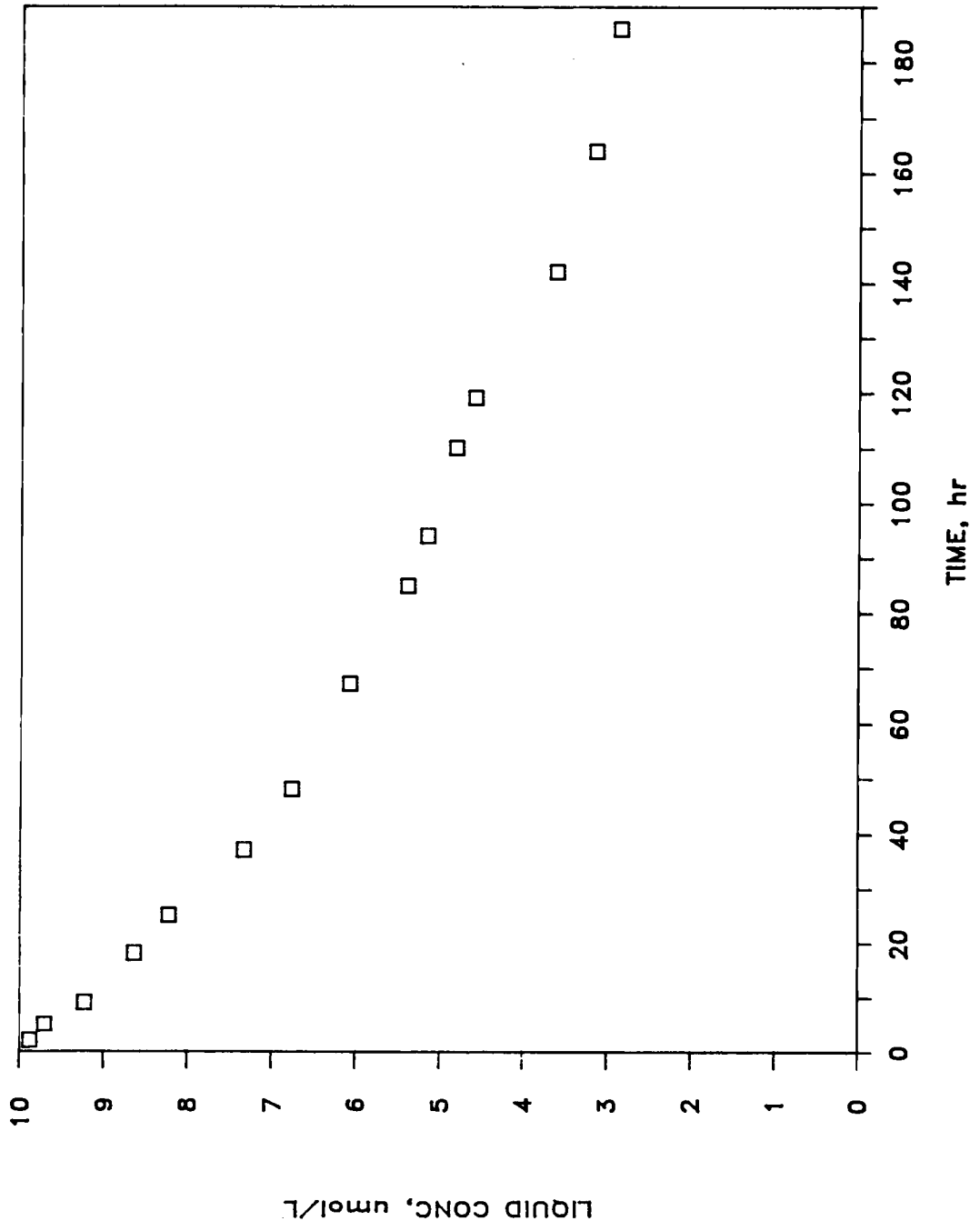

FIGURE 10 - Concentration of Chloroform in Reactor Liquid

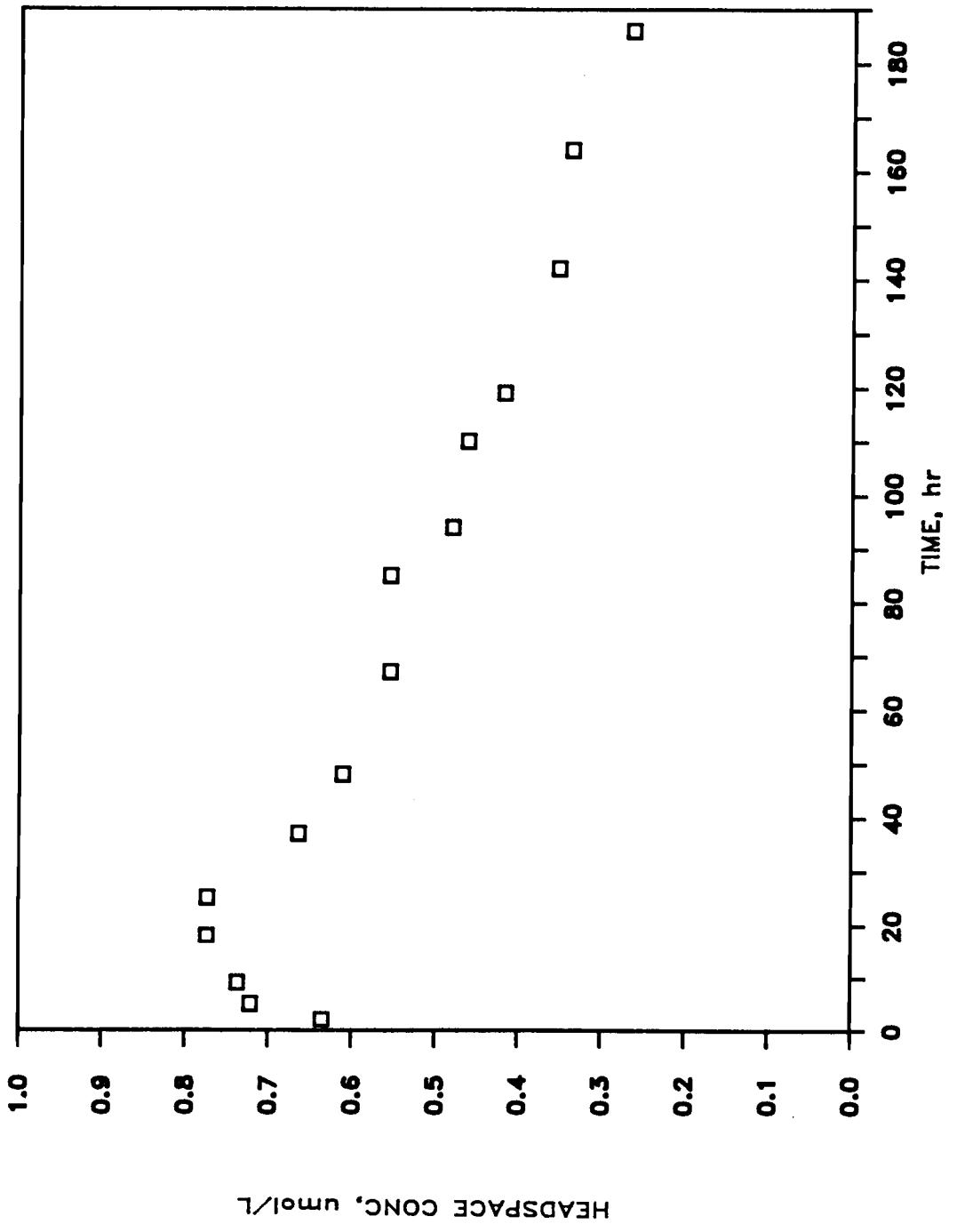

FIGURE 11 - Concentration of Chloroform in Reactor Headspace

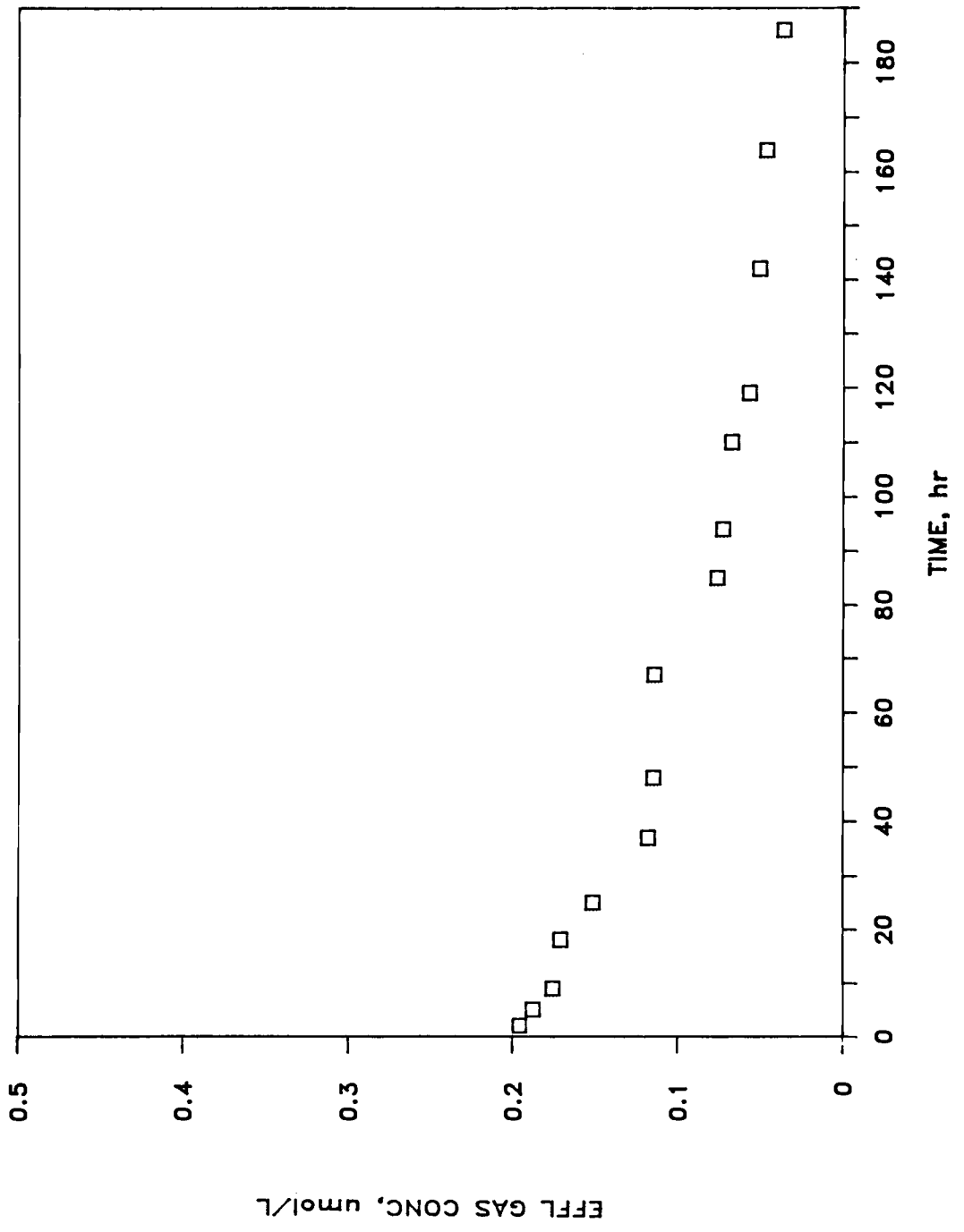

FIGURE 12 - Concentration of Chloroform in Effluent Gas

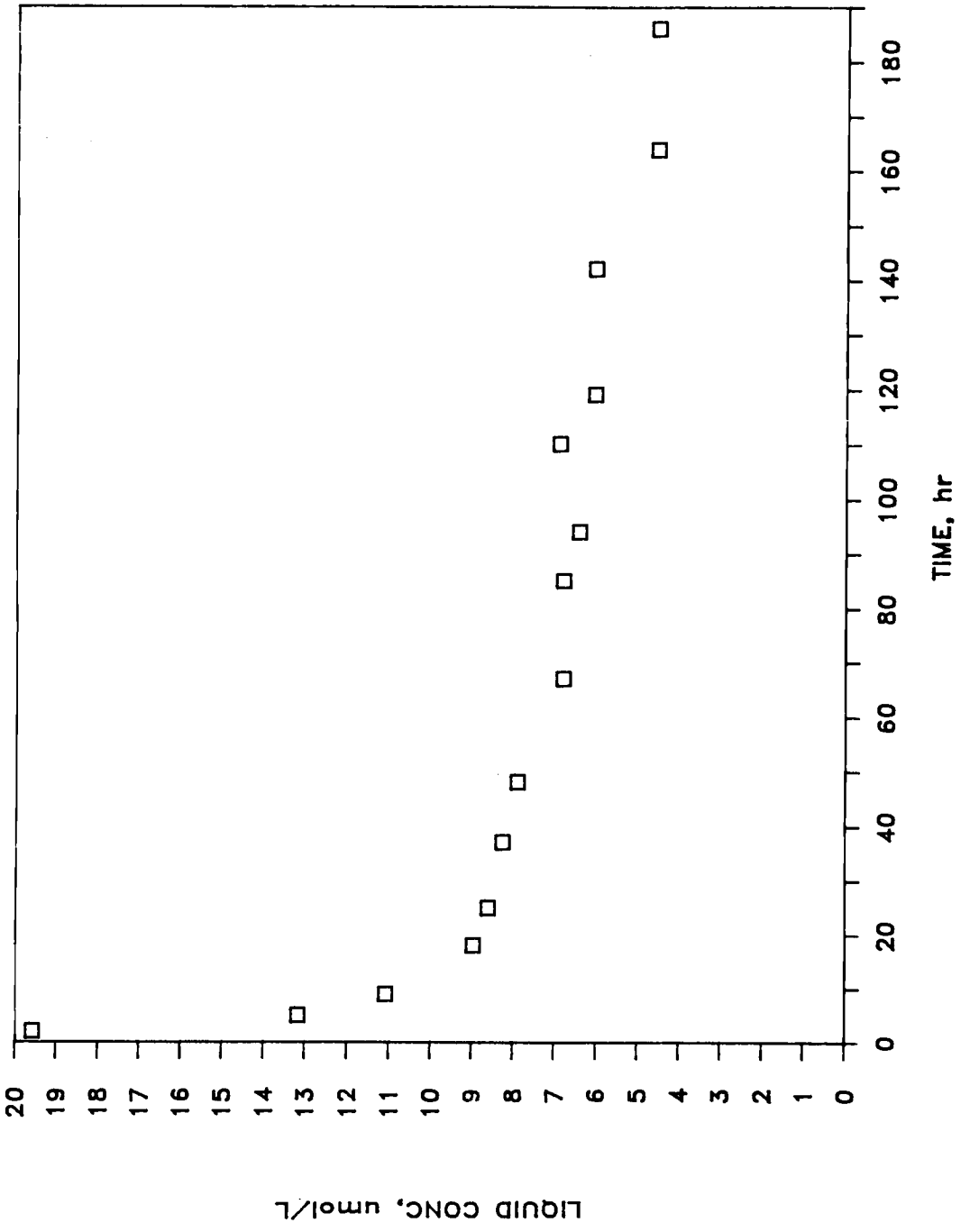

FIGURE 13 - Concentration of Carbon Tetrachloride in Reactor Liquid

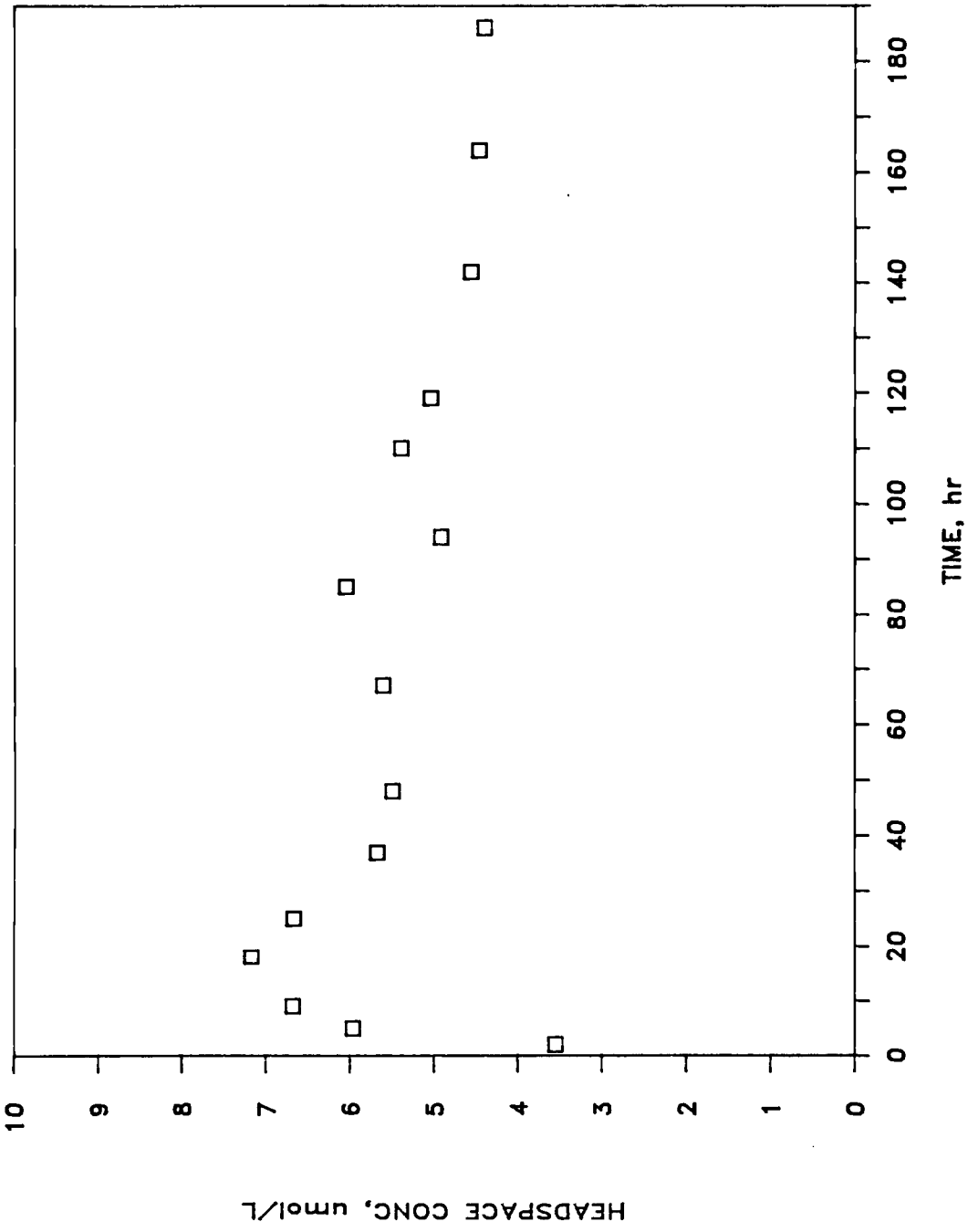

FIGURE 14 - Concentration of Carbon Tetrachloride in Reactor Headspace

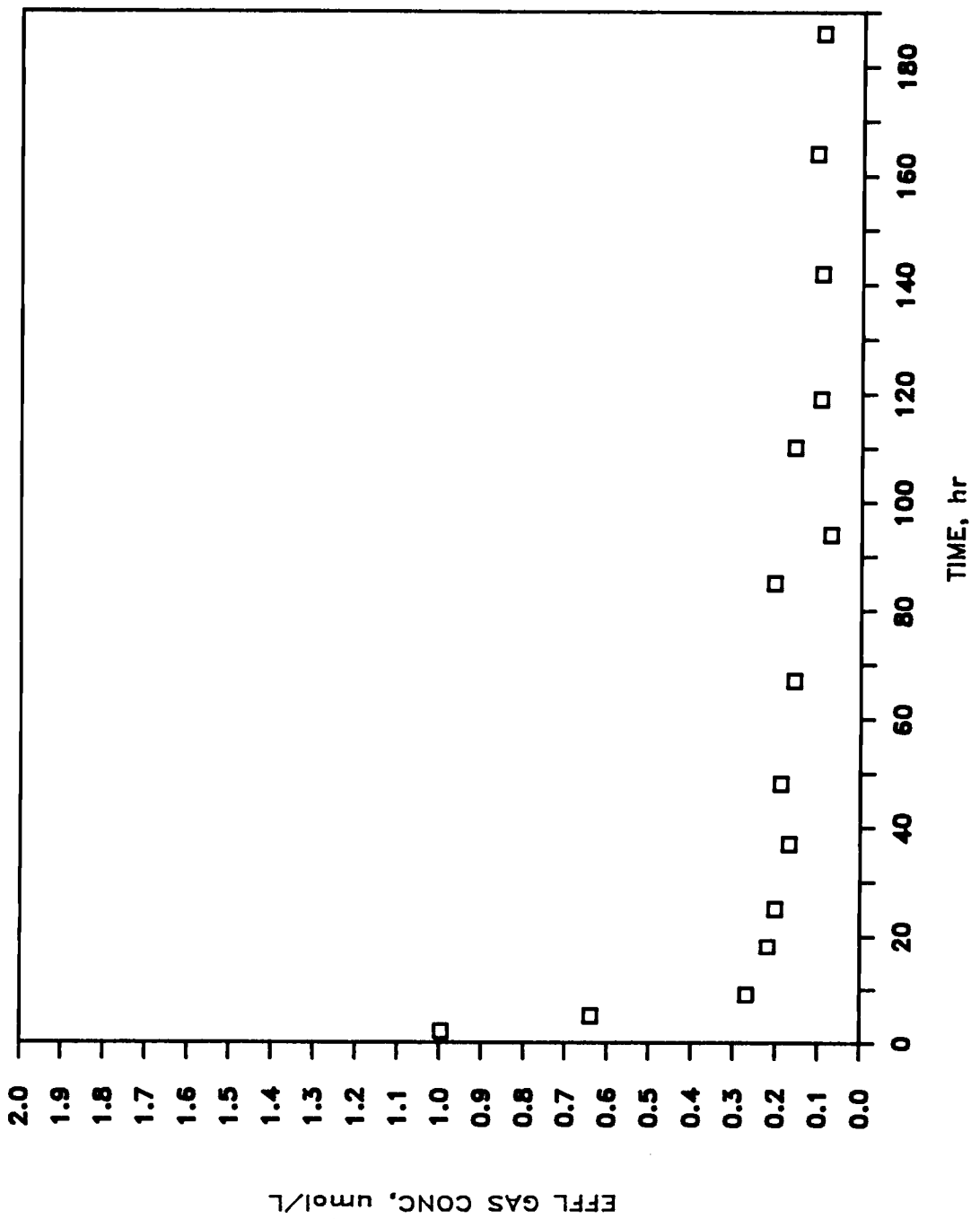

FIGURE 15 - Concentration of Carbon Tetrachloride in Effluent Gas

14 to 18 hours for the carbon tetrachloride. This illustrates the reason why headspace analysis techniques proved to be unworkable in this research. It is difficult to draw definite conclusions from these figures, because they do not really show how much of each compound was moving from one phase to another or how much was exiting the reactor with the gas compartment effluent gas.

Figures 16 through 18 are mass balances for each of the compounds. These figures show how much of each compound was present in the liquid phase and gas phase at any given time, as well as the cumulative amount of the compound that had exited the reactor in the gas compartment effluent gas up to that time. The difference between the total bar height at any time and the extrapolated height at time zero represents the cumulative degradation of the compound up to that time. These diagrams clearly show how much of the compound was degraded versus how much escaped through the membrane. Masses initially present were 20.6 micromoles, 19.6 micromoles, and 42.7 micromoles of methylene chloride, chloroform, and carbon tetrachloride, respectively. About 16.2 micromoles of methylene chloride were degraded, while about 4.4 micromoles escaped through the membrane. For chloroform, 3.3 micromoles were degraded while 10.3 micromoles escaped through the membrane. The carbon tetrachloride diagram would seem to suggest that a great deal of degradation took place during the first 24 hours of the test. However, it is more likely that this apparent rapid drop in the mass of carbon tetrachloride was due to some other effect, such as sorption of the compound to biomass and/or reactor components.

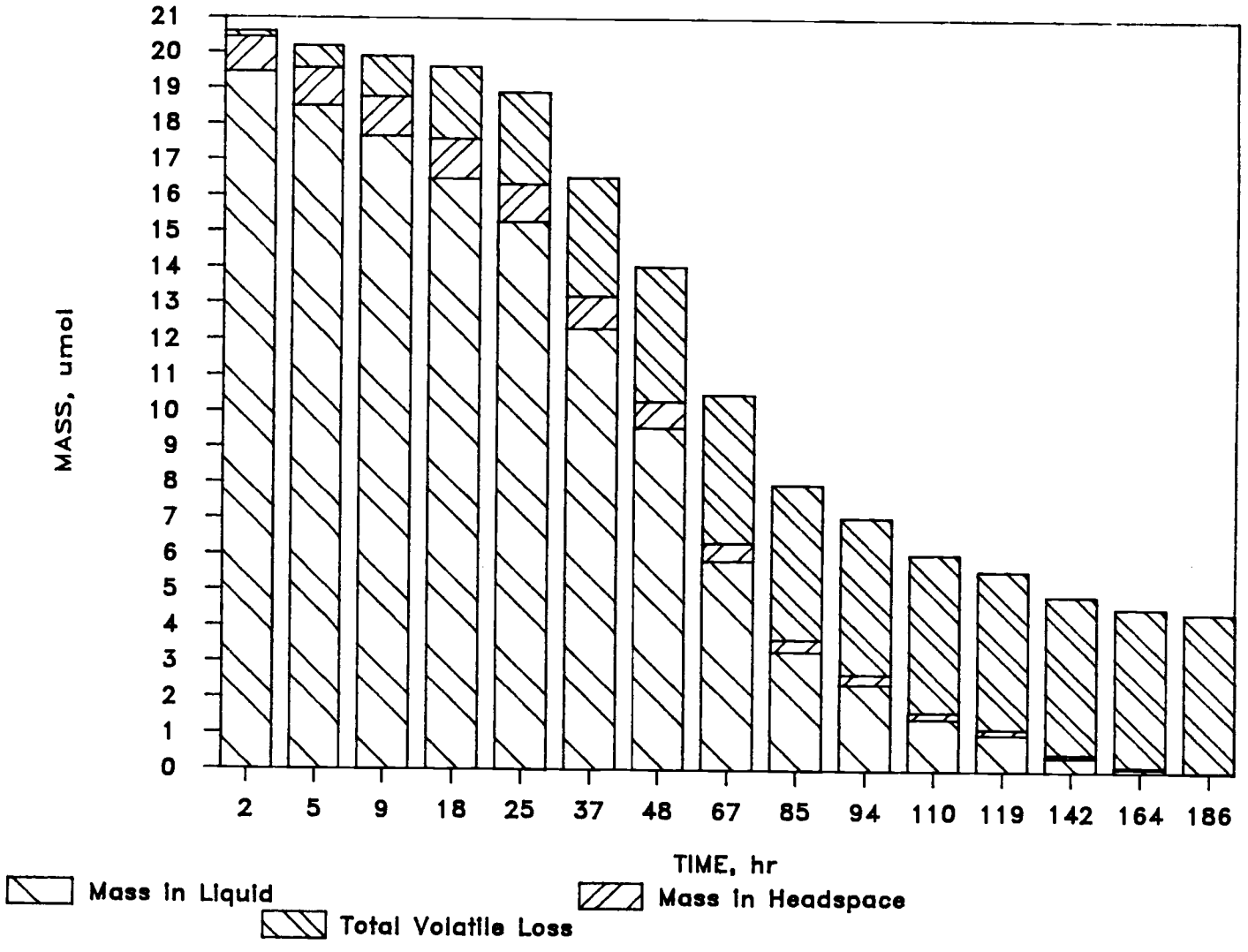

FIGURE 16 - Methylene Chloride Mass Balance

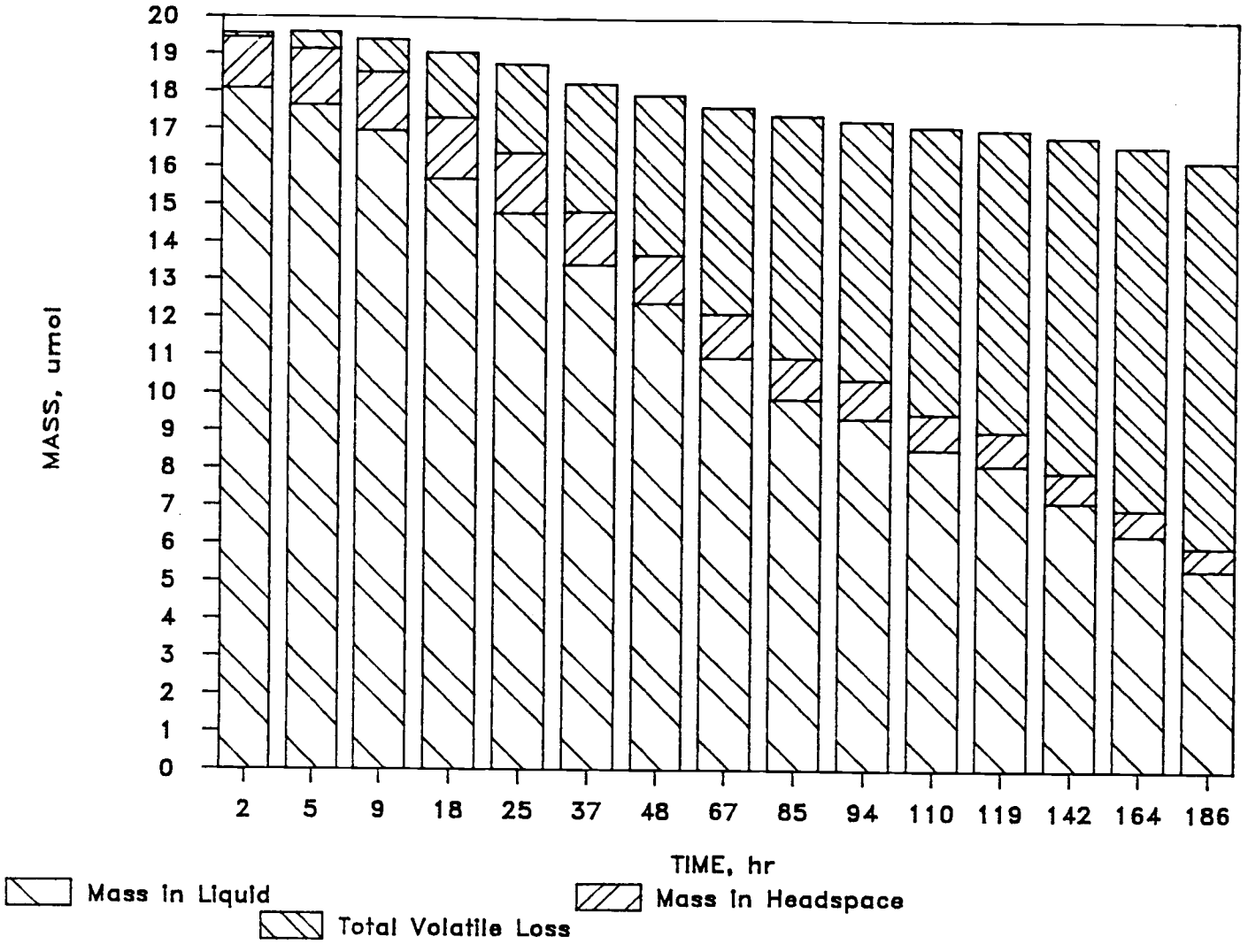

FIGURE 17 - Chloroform Mass Balance

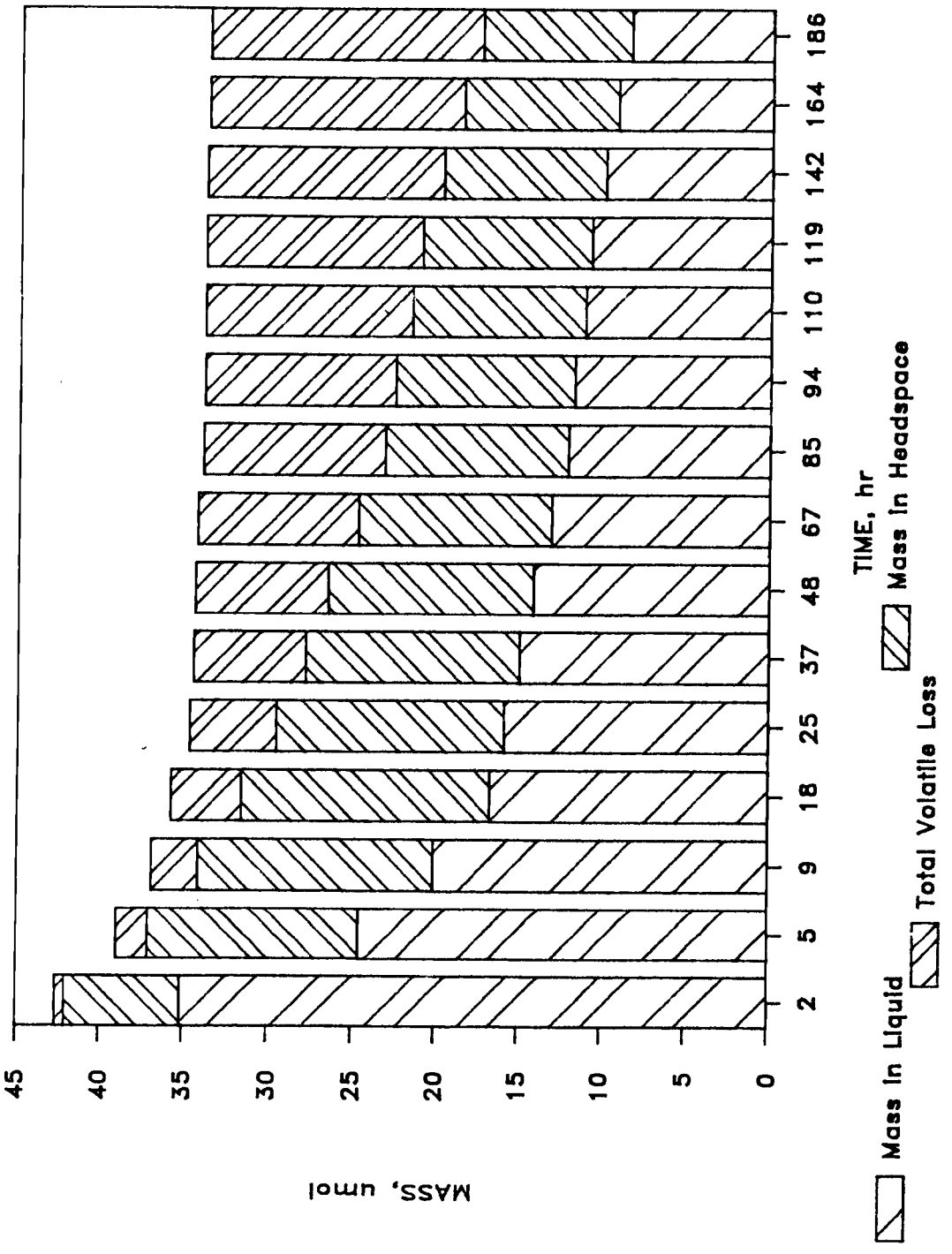

FIGURE 18 - Carbon Tetrachloride Mass Balance

While the data seem to suggest that about 8.9 micromoles of carbon tetrachloride were degraded and 16.3 micromoles lost through the membrane, it may be more reasonable to assume that of the 8.9 micromoles, about 1.2 micromoles were degraded and about 7.7 micromoles lost to initial sorption or other undefined effects. (This estimate was obtained by extrapolating back to time zero the trend of the curve between 25 and 186 hours.) Whether any sorbed compound could have been later degraded cannot be inferred from the data.

Pathways of Degradation and Enzyme Kinetics

The output from the GC was closely examined for metabolites in the hope that some information could be found regarding pathways of degradation. While small, unexpected peaks were ocasionally detected, there was no consistent, definite evidence of metabolites.

Figures 19, 20, and 21 are plots of degradation rates versus time for each of the compounds. It can be seen from these diagrams that, in general, methylene chloride degraded faster than chloroform, and that chloroform degraded faster than carbon tetrachloride. While it is not ideal to do so, performance of biofilms is often assessed using Monod kinetics. Figures 22, 23, and 24 show attempts to determine enzyme kinetic constants by the Hanes linearization method. It can be seen that no such determinations could be made for either chloroform or carbon tetrachloride. For methylene chloride, enzyme kinetic constants were determined by excluding data points during the first 25 hours

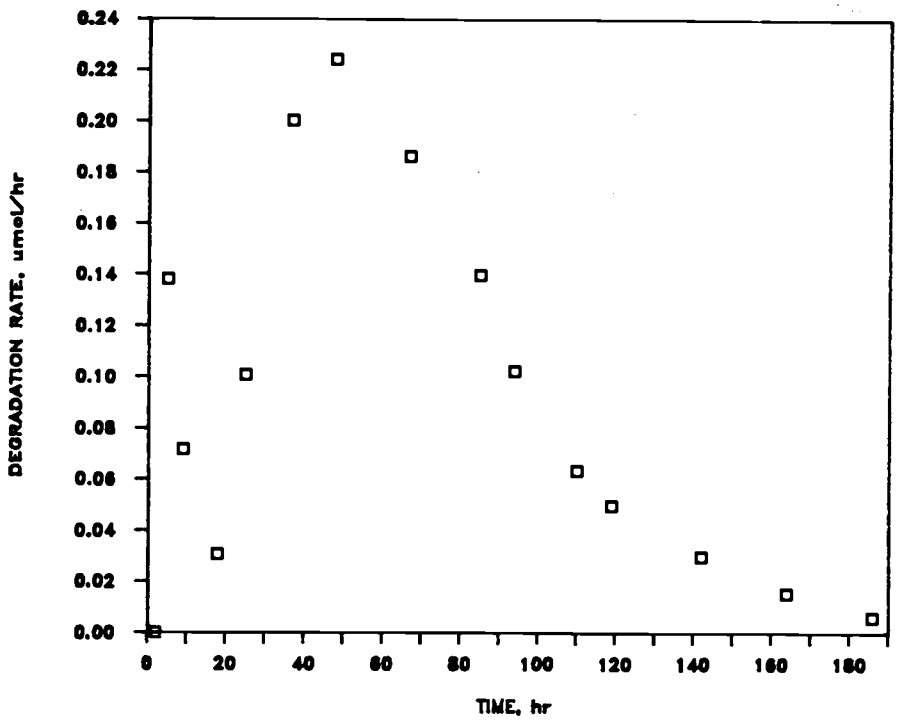

FIGURE 19 - Methylene Chloride Degradation Rates

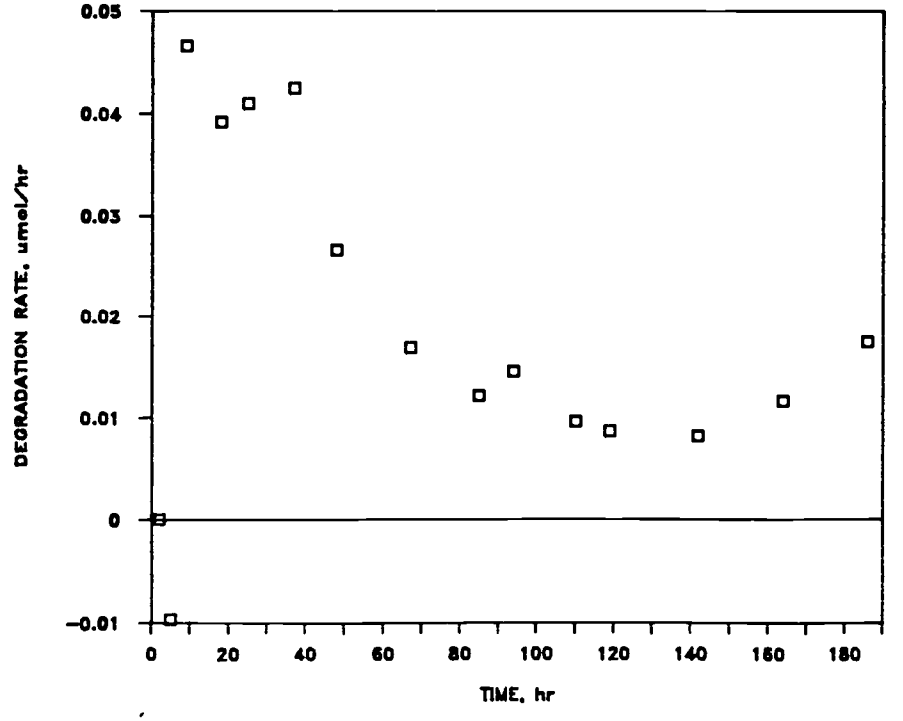

FIGURE 20 - Chloroform Degradation Rates

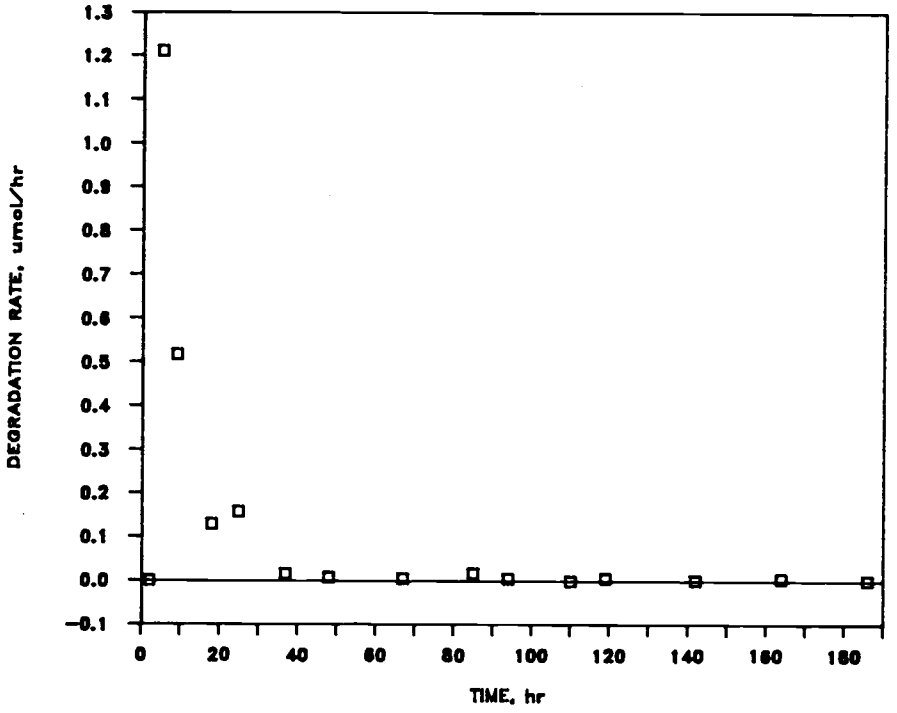

FIGURE 21 - Carbon Tetrachloride Degradation Rates

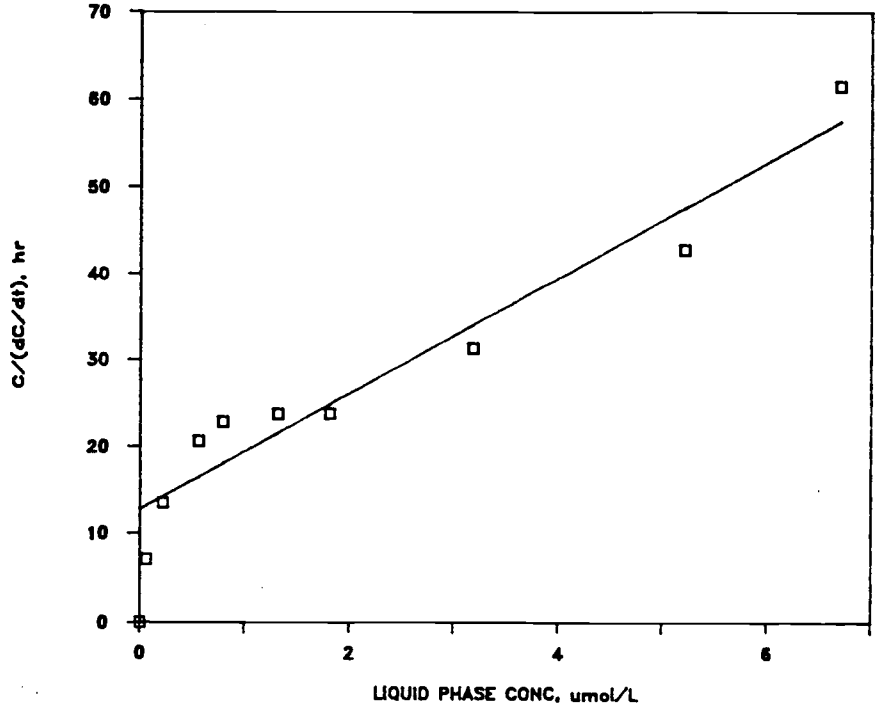

FIGURE 22 - Hanes Linearization of Methylene Chloride Degradation Data

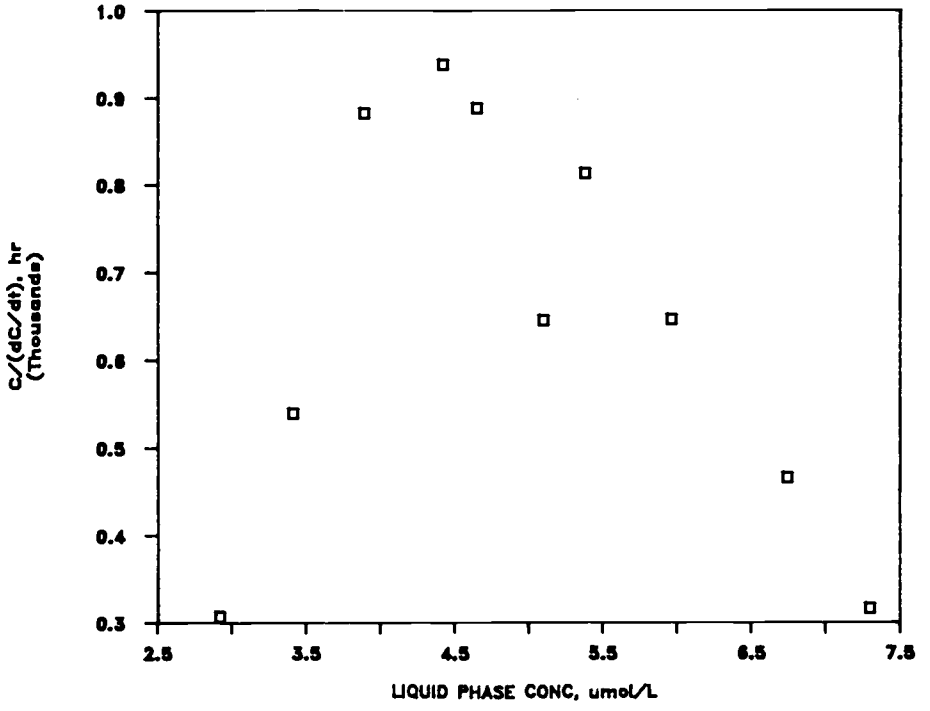

FIGURE 23 - Hanes Linearization of Chloroform Degradation Data

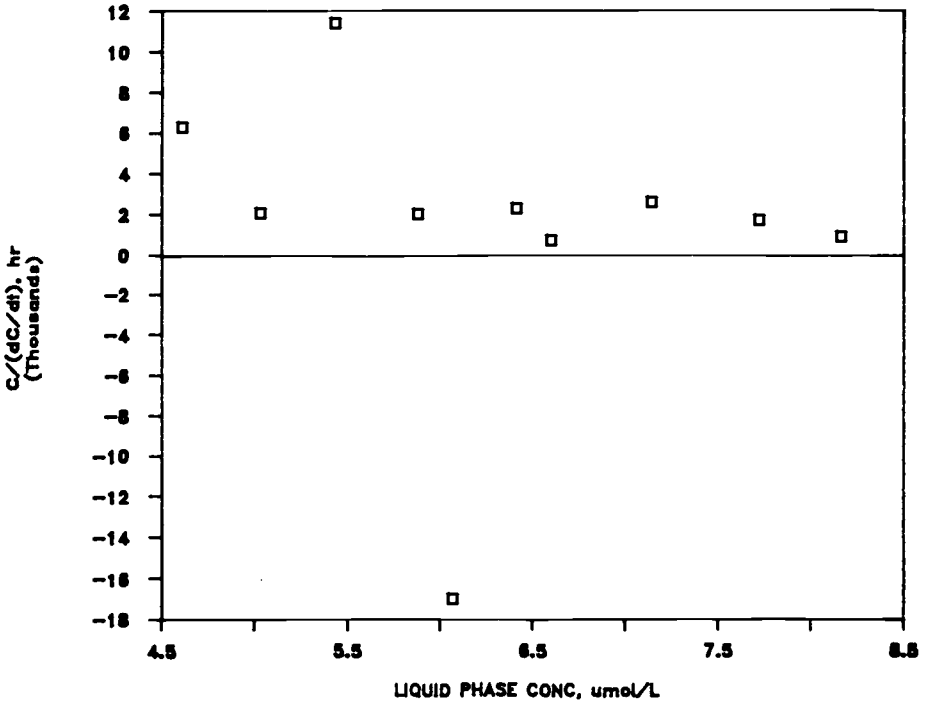

FIGURE 24 - Hanes Linearization of Carbon Tetrachloride Degradation Data

of the test when equilibrium between the gas and liquid phases had not yet been attained. The enzyme kinetic constants thus obtained were:

$$V_{max} = 0.150 \text{ umol/L-hr; and}$$

$$K_m = 1.90 \text{ umol/L}$$

DISCUSSION

General Performance of GPMS Biofilm

Whittenbury et al.(1970) reported a typical stoichiometry for methane oxidation by methylotrophs of:

$$1\ CH_4 + 1.0 \text{ to } 1.1\ O_2 \longrightarrow 0.2 \text{ to } 0.3\ CO_2$$

As mentioned previously, the molar oxygen to methane ratio observed in this research was about 0.54:1 at the beginning of the test and about 1.25:1 at the end. Also, the ratio of carbon dioxide produced to methane oxidized was about 0.2:1 at the beginning and about 0.15:1 at the end. Therefore, the results obtained with the GPMS biofilm are comparable to those obtained by Whittenbury. Working with obligate methylotrophs Methylosinus trichosporium OB3b and Methylococcus capsulatus CRL M1, Hou et al. (1979a) observed methane oxidation rates of 19.2 and 30 mmol of methane per day per gram of biomass, respectively. These figures compare favorably to the 28.8 mmol per day per gram of biomass observed during this work. These comparisons indicate that the performance of the methylotrophic GPMS biofilm used during this study was equivalent to the performance of methylotrophic bacteria used in other types of systems.

It is interesting to note that biofilm oxygen utilization was lowest during the time that nitrogen production was highest.

Denitrification ability has been documented for methylotrophs (Kosaric and Zajic, 1974), with a reported stoichiometry of:

$$3\ CH_4 + 2\ NO_3^- \longrightarrow 3\ CO_2 + N_2 + 6\ H_2 - 129\ Kcal$$

Whittenbury et al. (1970) found some methylotrophs capable of reducing nitrate to nitrite, but incapable of using nitrate as an alternative electron acceptor to oxygen in an anaerobic environment.

It appears that significant methylotrophic denitrification activity may have occurred during the first three or four weeks of the test period. This would explain the low oxygen:methane ratio observed during that time in that the methylotrophs would have been obtaining supplemental electron acceptors from denitrification. However, why would this denitrification activity have disappeared? Another possible explanation for the initial denitrification activity could have been the presence of denitrifiers in the original seed. Denitrifiers present in the seed could have remained viable in the reactor by consuming methanol or other metabolites produced by the methylotrophs. Again, the reason for their disappearance would be a mystery. Also, this hypothesis would not explain the low oxygen:methane ratio evident during the first half of the test period.

A comparison of the oxygen transfer data obtained during the oxygen transfer testing to that obtained after the biofilm was established on the membrane discloses that some changes may have taken place. During the oxygen transfer testing, it was indicated

that about 2.1 mg of oxygen could be transferred per day per square centimeter of membrane area. With the biofilm on the membrane, oxygen transfer was about 2.0 mg/cm^2-day at the beginning of the test period but increased to about 8.6 mg/cm^2-day at the end of the test period. This represents over a fourfold increase in oxygen transfer through the membrane and suggests that either the properties of the membrane changed with time, or that the bacteria may have begun to penetrate the membrane and actually began to consume oxygen before it had passed all the way through the membrane. It may be possible that during the first half of the test period, the biofilm was oxygen limited, causing the methylotrophs to rely on their denitrification capabilities. With time, they began to grow more deeply into the membrane, giving more direct access to the oxygen in the gas compartment. As oxygen became more readily available, the need to perform the less efficient denitrification activity gradually decreased.

Figures 25 through 28 show the results of an electron balance that was performed on the data. The electron balance was obtained using stoichiometry for overall synthesis/energy reactions determined by energetics analyses (see Appendix D). A linear regression analysis of the data in Figure 25 indicates that about 19 mmol of electrons per day were being accepted by oxygen at the beginning of the test period (r^2 = 0.554), while a similar analysis of the data in Figure 26 suggests that about 62 mmol of electrons per day were being accepted as a result of denitrification activity (r^2 = 0.287). At the end of the test period, it appears that about

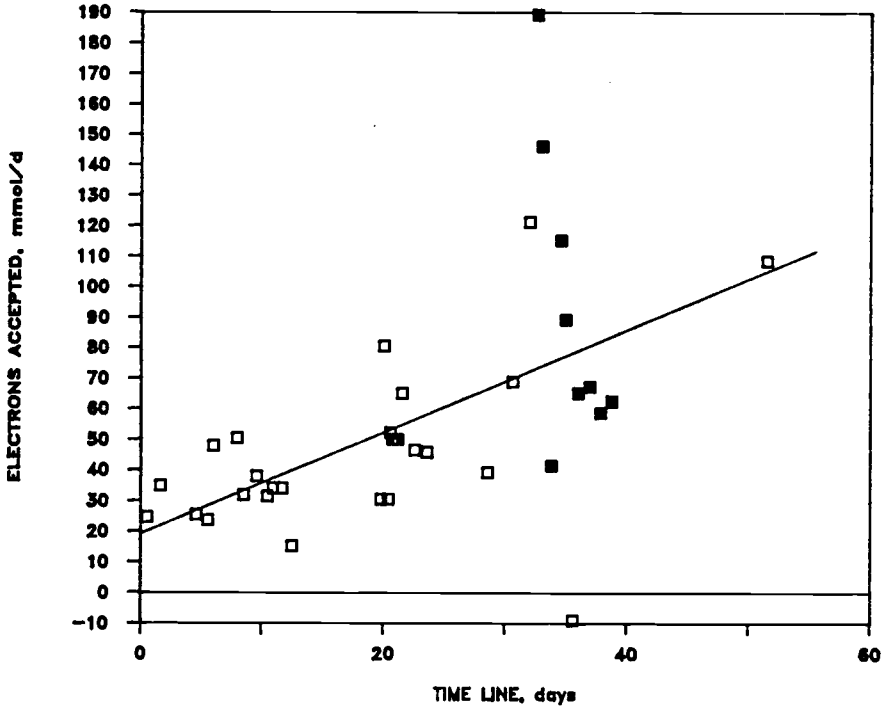

FIGURE 25 - Electrons Accepted by Oxygen

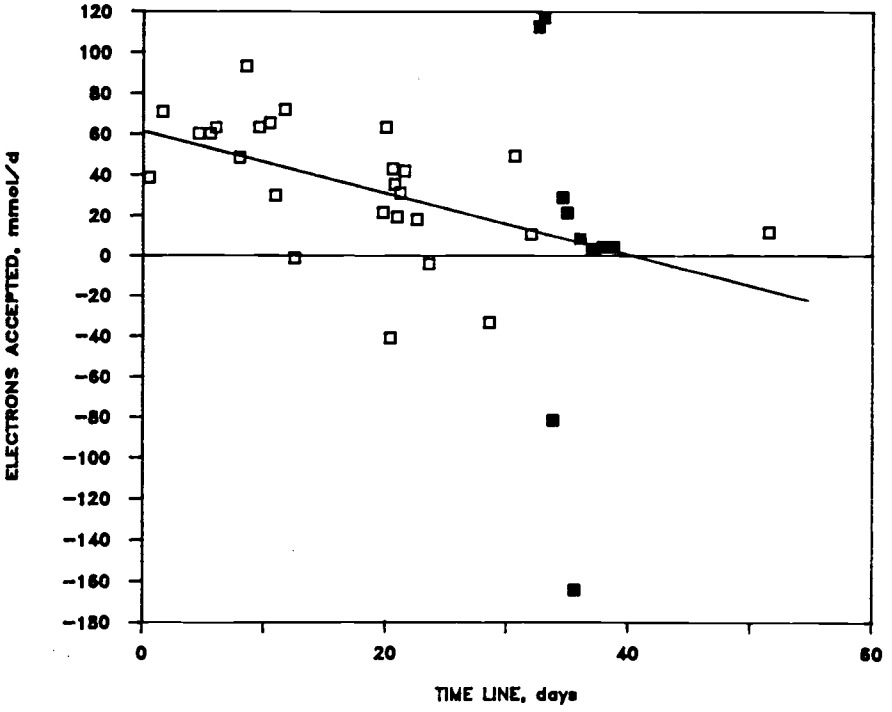

FIGURE 26 - Electrons Accepted by Nitrate

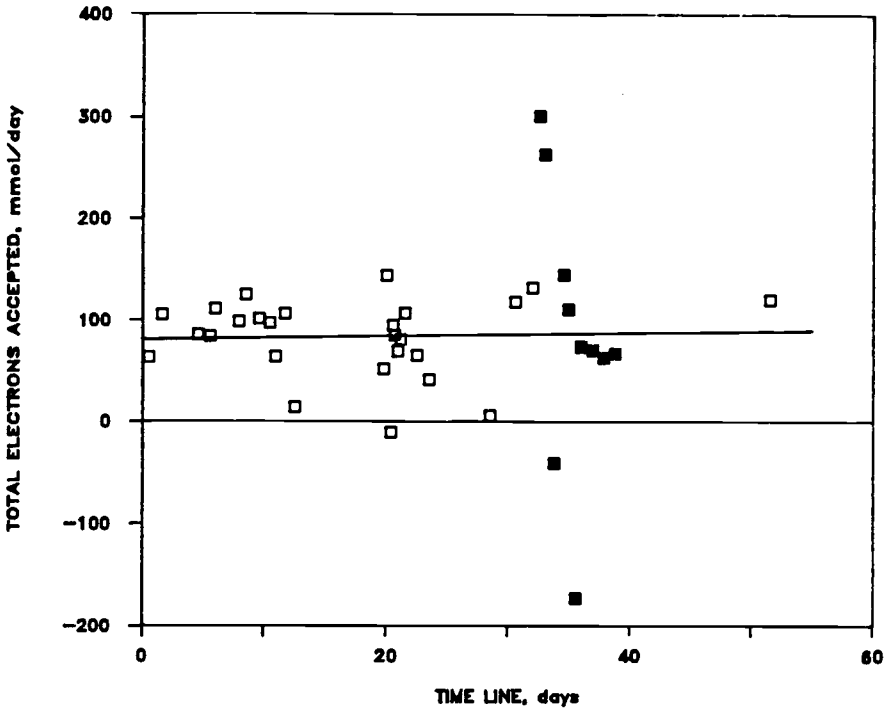

FIGURE 27 - Total Electrons Accepted by Oxygen and Nitrate

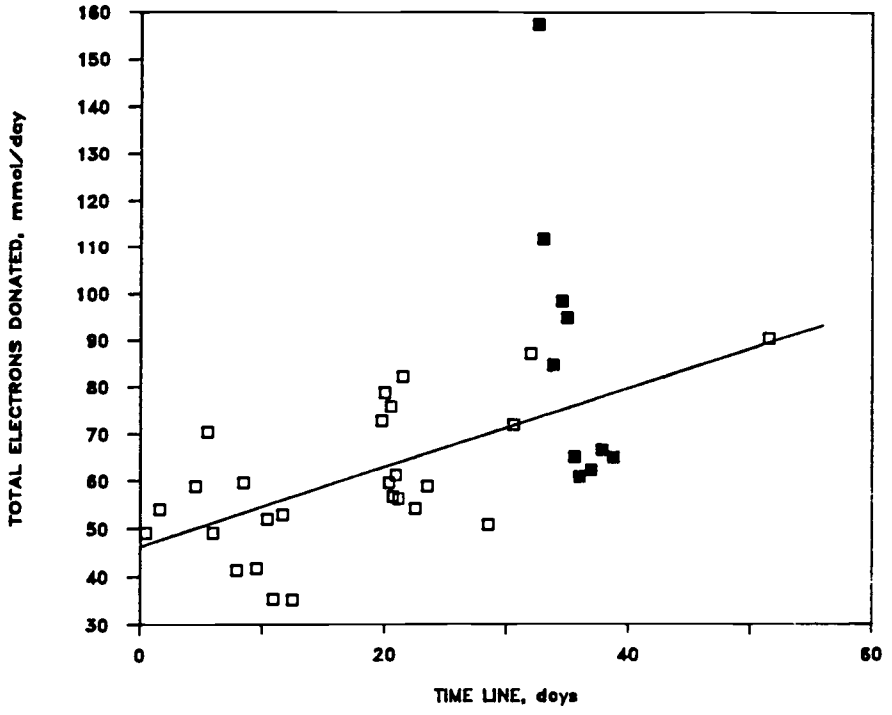

FIGURE 28 - Electrons Donated by Methane

105 mmol of electrons per day were being accepted by oxygen while the number of electrons that were being accepted as a result of denitrification activity had dropped to about -18 mmol per day. (It is questionable whether the negative value for electrons accepted by denitrification is accurate. Perhaps a straight-line curve fit is not representative of the actual conditions within the reactor.) Using the straight line fit, the linear regression analysis suggests that a total of about 81 mmol of electrons per day were being accepted at the beginning of the test period and about 87 mmol per day were being accepted at the end of the test period ($r^2 = 0.001$). This is shown in Figure 27. If the straight line fit for electrons accepted by denitrification is discounted, the total electrons accepted could have been about 105 mmol per day at the end of the test period. Although there is a great deal of scatter in the data, it can be seen from these figures that while the daily quantity of electrons accepted may have increased only slightly over the test period, a trend toward more use of oxygen and less dentrification is definitely apparent.

Figure 28 indicates that about 46 mmol of electrons per day were being donated by methane oxidation at the beginning of the test period and that about 89 mmol per day were being donated by methane at the end of the test period ($r^2 = 0.392$). Inasmuch as the number of electrons donated must be equal to the number of electrons accepted, it would seem that another electron donor must have been present at the beginning of the test period. It could be possible that some autotrophic activity was taking place, or that other

organisms were using nitrate as an electron acceptor along with an unidentified electron donor. It is possible that a relatively small number of other organisms could have used EDTA or ammonia present in the nutrient media as electron donors. These organisms could have died out over a period of time as methane was the only carbon source being continuously supplied to the reactor. It must be emphasized that the electron balance is a rough approximation. A great deal of scatter exists in the data, and the reaction stoichiometry is somewhat uncertain (and may have changed over the test period). However, the evidence of early denitrification and the gradual changeover to more use of oxygen as the primary electron acceptor seems clear. There seems to have been a changeover from reliance on denitrification activity to provide electron acceptor capacity to reliance on oxygen as the primary electron acceptor.

Therefore, it is suggested that the scenario described above did in fact occur, i.e, that the methylotrophs supplemented the amount of oxygen that could be transferred through the membrane and satisfied their electron acceptor requirements by denitrification early in the test period. With time, the methylotrophs began to penetrate and grow within the interstitial spaces of the membrane and effectively shortcut the oxygen transfer capability of the membrane. Thus what appears to be an increase in the oxygen transfer capability of the membrane is actually caused by the bacteria finding a more effective way to "get at" the oxygen that was on the other side. Because the membrane is currently being used

in a series of further GPMS biofilm tests, it is not possible at this time to investigate the validity of this idea by inspecting the interior of the membrane.

Another interesting point is that during the time that the chlorinated compounds were present in the reactor (days 32-39), Figures 2 through 5 show that changes in gas consumption/production seem to have occurred. Carbon dioxide production and oxygen utilization seem to have increased, as might be expected due to the additional oxidative activity that was evidently transpiring. However, methane utilization and nitrogen production also seem to have increased. While the increase in nitrogen could be explained in light of the preceeding discussion, the increase in methane utilization is a bit puzzling. It is almost as though the presence of the chlorinated compounds provided some stimulus to the activity of the methylotrophs. Due to the variability of the data, it is not possible to advance this discussion beyond the realm of speculation. No explanation can be given for the apparent increase in methane usage that occurred while the chlorinated compounds were present in the reactor.

Degradation of Chlorinated Compounds

Degradation of chlorinated methanes by the GPMS biofilm also appears to have been similar to that observed by researchers using methylotrophs with other types of systems. Working with soluble MMO from *Methylococcus capsulatus* (Bath), Colby et al. (1977) observed that monochloromethane and methylene chloride were oxidized as

quickly as methane itself. Chloroform was oxidized more slowly and carbon tetrachloride was not oxidized at all. Colby was not able to detect any volatile metabolites by gas chromotography and suggested that this was due to the instability of the 1-substituted methanol derivatives. No methylotrophic degradation of these compounds was obtained under anaerobic conditions. Colby concluded that up to two chlorine substitutions did not effect the ability of enzyme to oxidize the compound, that with more than two substitutions the rate of oxidation was slowed, and that the enzyme was not able to oxidize fully substituted methanes. Working with soluble MMO from Methylobacterium sp. strain CRL-26, Patel et al. (1982) also observed that monochloromethane and methylene chloride were oxidized faster than chloroform, but that they were not oxidized as rapidly as methane. Formaldehyde was detected as a metabolite of monochloromethane, but no metabolites were detected for methylene chloride or chloroform. Patel did not test for oxidation of carbon tetrachloride.

As in the two studies cited above, methylene chloride degradation by the methylotrophic GPMS biofilm proceeded faster than chloroform degradation. Little or no carbon tetrachloride appears to have been oxidized. No metabolites were detected. Enzyme kinetic constants were determined for the degradation of methylene chloride, but they could not be determined for chloroform because the rate of chloroform degradation varied a great deal. (Enzyme kinetic constants for degradation of these compounds have not been

previously reported.) At least part of this variation appears to have resulted from the concurrent degradation of the methylene chloride.

It is interesting to note that when the methylene chloride concentration decreased to a very low level, it appears that the chloroform degradation rate increased (Figures 7 and 20). Unfortunately this was not detected when the test was in progress, and the test was ended before this could be verified. Sequential degradation of substrates has been shown to occur in batch cultures under nutrient-sufficient conditions (Hamer et al., 1985). This usually applies in situations where different enzymes are used to degrade the substrates, however a similar situation could exist here. Perhaps when both methylene chloride and chloroform are present, there is a preference on the part of the enzyme for methylene chloride, causing chloroform to be degraded more slowly than it would be if the methylene chloride were not present. If this were true, analysis of the enzyme kinetics of chloroform degradation both with and without methylene chloride being present should show a competitive inhibition effect caused by the methylene chloride. Methylene chloride degradation could also be slowed by the presence of chloroform, but not as significantly.

It was not possible to evaluate dechlorination by measuring chloride release because of the high chloride concentration in the nutrient solution. The nutrient solution contained about 65 mg/L of chloride ion. For the amounts of chlorinated compounds degraded, a total chloride release of only about 0.9 mg/L would have been

expected. This would not have been detectable. For dehalogenation of substituted n-alkanes by oxygenase enzymes, Yokota et al. (1986) suggested that dechlorination of a terminal chlorine atom occurs according to the following reaction sequence:

$$R\text{-}CH_2Cl \xrightarrow[\text{oxygenase}]{} R\text{-}CHClOH \xrightarrow[\text{spontaneous}]{} R\text{-}CHO \xrightarrow[\text{dehydrogenase}]{} R\text{-}COOH$$

Yokota's presentation agrees with Colby's position regarding why he was not able to detect volatile metabolites in that any volatile metabolites would have decomposed spontaneously as they were formed. The same could be true for the GPMS biofilm. Also, since degradation occurs faster for compounds with fewer chlorine atoms as shown for monochloromethane, methylene chloride, and chloroform, any dechlorination activity should cause the metabolites to degrade more rapidly than the parent compound. Thus in a batch reactor, one would not expect to see accumulations of significant concentrations of metabolites. Because the reactor liquid was essentially oxygen free, it could be possible that reductive dechlorination could also occur with the GPMS biofilm if the necessary organisms were present. It is possible that a small amount of reductive dechlorination occurred during this study. However, metabolites would still not be expected to be visible by GC analysis inasmuch as compounds with fewer chlorines were oxidized more rapidly than more fully substituted compounds. While some reductive dechlorination could have occurred, it is suggested that the reactions proposed by Yokota

probably apply for most of the dechlorination activity by the GPMS biofilm under the conditions of this study.

An issue of significant concern during this study was the minimization of unaccounted losses of the volatile compounds from the reactor liquid compartment--both from the headspace and from the reactor liquid itself. Based on the following reasoning, it is felt that unaccounted volatile losses were not significant. Due to the much higher concentration of carbon tetrachloride in the reactor headspace (Figures 8, 11, and 14), there would have been more tendency to lose carbon tetrachloride than to lose the other two compounds from the headspace. Even though the diffusion coefficients of methylene chloride and chloroform are roughly 1.26 and 1.05 times greater, respectively (Smith and Bomberger, 1978), than that of carbon tetrachloride, the concentration of carbon tetrachloride in the headspace was approximately an order of magnitude greater than the other two compounds. Therefore, based on diffusion theory, if compounds were leaking from the headspace one would expect more loss of carbon tetrachloride than of either methylene chloride or chloroform. Similar reasoning would apply with regard to losses from the liquid itself. The carbon tetrachloride concentration in the liquid was roughly equal to, or greater than, the concentrations of the other two compounds. Therefore, any losses of carbon tetrachloride from the liquid should have been approximately equal to or greater than losses of the other two compounds.

The mass balance on carbon tetrachloride does not indicate any significant volatile loss from the reactor headspace or liquid. Although it was not planned, the fact that the carbon tetrachloride was only minimally degraded (if at all) allowed it to function almost like a conservative, volatile tracer indicating that no significant unaccounted losses of the compounds occurred.

SUMMARY AND CONCLUSIONS

Based on the results of this study, the following conclusions may be drawn:

1. It is possible to grow a methylotrophic biofilm on a gas-permeable membrane with transfer of methane and oxygen through the membrane;

2. The Teflon/nylon material manufactured by W.L. Gore & Associates, known as Goretex, is an acceptable membrane material capable of supporting this type of biofilm;

3. Approximate consumption and production of gases by a methylotrophic GPMS biofilm can be expected to be:

Methane Utilization	28.8 mmol/d-g of biomass
	0.46 mg/d-g of biomass
Oxygen Utilization	36 mmol/d-g of biomass
	1.1 mg/d-g of biomass
Carbon Dioxide Production	0.15 to 0.2 mmol/mmol CH4
Nitrogen Production/Utilization	-1.6 to 5.8 mmol/mmol CH4
COD Consumption	5.5 mg/d-cm_2 of biofilm

It also appears that a methylotrophic GPMS biofilm may be able to supplement available oxygen by means of denitrification;

4. A GPMS biofilm can degrade both methylene chloride and chloroform, with methylene chloride being degraded more rapidly than chloroform;
5. Carbon tetrachloride is not degraded appreciably by this type of biofilm;
6. In a batch reactor, no significant metabolites are detectable from the degradation of methylene chloride and chloroform;
7. The presence of chlorinated compounds may cause increases in methane and oxygen utilization as well as increases in carbon dioxide and nitrogen production.

SIGNIFICANCE AND SUGGESTIONS FOR FUTURE RESEARCH

This research has demonstrated that it is possible to grow a methylotrophic GPMS biofilm and that the performance of the biofilm is similar to that previously observed by researchers working with methylotrophs in other types of systems. This suggests that a methylotrophic GPMS biofilm could be effective in treating an array of recalcitrant compounds. Based on the results of this study, one may expect that compounds which have previously been shown to be vulnerable to methylotrophic degradation could be effectively treated using a methylotrophic GPMS biofilm. This approach is advantageous in that it is effective in oxidizing low level concentrations of recalcitrant organics and does not appear to be vulnerable to biomass loss and other problems associated with sloughing.

Inasmuch as the possible application of methylotrophic GPMS biofilms in treatment of industrial wastewaters and/or contaminated groundwaters appears promising, further research is needed. Certain aspects of the system warrant further investigation, as well as possible expansion of its application capabilities. For example, one item that was noticeable during this work was that significant quantities of the compounds were lost by diffusion through the membrane, exiting with the effluent gas. It would be helpful to reduce this loss either by making the gas compartment larger to increase its retention time and/or providing a way whereby the effluent gas could be recycled. Either of these modifications, or a

combination of them, would be beneficial in a number of respects. First, more efficient utilization of the gases being supplied to the gas compartment would be encouraged. On the average, only about 6% of the gas compartment influent gas was actually used by the biofilm. This was mainly because the system was so small that much more gas flow had to be used than was actually needed just to be able to measure it reliably. Second, providing a longer gas retention time and/or recycling the effluent gas could reduce the amount of volatile compounds exiting through the membrane by keeping them exposed to the methylotrophs for a longer period of time. This would not be so important for methylene chloride and other compounds that degrade rapidly, but it would be very important for more slowly degrading compounds such as chloroform.

Another modification that would be helpful would be to make the membrane surface area larger with respect to the liquid volume being treated. In this work a total membrane surface area of 81.1 cm^2 supported a liquid volume of 1.84 L, or about 23 ml of liquid per square centimeter of membrane surface area. This means that the effective biomass concentration was about 325 mg of biomass per liter of liquid in the reactor. This is approximately an order of magnitude less than is typical in conventional suspended-growth wastewater treatment systems. While measurements of MMO enzyme concentration were not made during this testing, increasing the biomass:liquid volume ratio should increase the enzyme concentration and increase the speed at which the chlorinated compounds are

degraded. Speeding up the degradation of the volatile compounds would also help decrease the amounts escaping through the membrane. In doing this, care would need to be taken to ensure that ample nutrient levels were maintained in the reactor liquid.

Syntrophic associations of bacteria in mixed culture are well documented. It would be very interesting to determine if it would be possible to establish a mixed culture of methylotrophs and methanogens in a single reactor. In a mixed culture of methane-oxidizing and hydrogen-utilizing bacteria established in a closed system, gaseous degradation products were recycled and it was shown that the hydrogen utilizers were effective in consuming all of the carbon dioxide produced by the methane oxidizers (Morinaga et al., 1980). If a similar approach could be used and both methylotrophs and methanogens established in a single reactor, the methane produced by the methanogens could perhaps be used as a carbon source by the methylotrophs. This would not be possible using conventional technologies, but an advantageous characteristic of the GPMS biofilm is that it allows aerobic bacteria to grow on the membrane while the bulk liquid can remain essentially anaerobic. Using a GPMS biofilm it is possible to grow multiple layers of bacteria with the bacterial cultures transitioning from strict aerobes nearest the gas compartment, through a layer of denitrifiers or microaerophiles, and ultimately to anaerobes adjacent to the bulk liquid (Timberlake, 1985). Not only could this provide methane to help sustain the methylotrophs, but it could also take advantage of the documented capabilities of anaerobic bacteria to dechlorinate more fully

substituted compounds that are more resistant to oxidation by methylotrophs (Bouwer et al., 1981, Gossett, 1985). If this type of system could be established, perhaps an effective "one-two punch" combining the capabilities of both the methylotrophs and the anaerobes could be used to effectively degrade many recalcitrant compounds that currently present problems in the environment.

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APPENDICES

APPENDIX A

Gas Data Spreadsheet

Oxygen, Methane, & COD Balances

Note: Gas volumes, flows, pressures, and temperatures are measured values. All other values are calculated.

	Month	Day	Month	Day	Month	Day	Month	Day
Date:	8	20	8	21	8	24	8	25
Time:	1230		1500		1400		1330	
	In	Out	In	Out	In	Out	In	Out
Oxygen Volume:	90.02	90.95	107.28	110.57	120.82	123.83	72.80	74.04
Methane Volume:	106.09	105.63	78.83	79.15	114.10	113.73	114.10	114.26
Nitrogen Volume:	2.75	4.68	2.83	5.83	3.38	6.48	1.92	3.89
Carb Diox Volume:	0.00	2.26	0.00	1.46	0.00	1.24	0.00	0.00
Total Volume:	198.86	203.52	188.94	197.01	238.30	245.28	188.82	192.19
% Oxygen:	45.27	44.69	56.78	56.12	50.70	50.49	38.56	38.52
% Methane:	53.35	51.90	41.72	40.18	47.88	46.37	60.43	59.45
% Nitrogen:	1.38	2.30	1.50	2.96	1.42	2.64	1.02	2.02
% CO2:	0.00	1.11	0.00	0.74	0.00	0.51	0.00	0.00
Temp, K:								
Gas Flow, ml/min:	6.93	6.93	7.82	7.82	7.98	7.98	9.80	9.80
Gas Press, in H2O:	12.00	4.50	12.60	4.50	13.00	4.50	14.40	4.50
Obs Vol Change, ml:								
Act Vol Change, ml:								
Oxygen, ml/d:	4651	4509	6592	6390	6012	5866	5634	5497
Methane, ml/d:	5481	5237	4844	4574	5678	5387	8829	8483
Nitrogen, ml/d:	142	232	174	337	168	307	149	289
CO2, ml/d:	0	112	0	84	0	59	0	0
Oxygen, mmol/d:	187.1	181.4	265.1	257.0	241.8	235.9	226.6	221.1
Methane, mmol/d:	220.5	210.6	194.8	184.0	228.4	216.7	355.1	341.2
Nitrogen, mmol/d:	5.7	9.3	7.0	13.6	6.8	12.3	6.0	11.6
CO2, mmol/d:	0.0	4.5	0.0	3.4	0.0	2.4	0.0	0.0
COD, mg/d:	8123.2	7677.0	3984.5	3550.4	6877.8	6318.1	15478.2	14761.5
O2 in-out, mmol/d:	5.7		8.1		5.9		5.5	
CH4 in-out, mmol/d	9.8		10.8		11.7		14.0	
N2 in-out, mmol/d:	-3.6		-6.6		-5.6		-5.6	
CO2 in-out, mmol/d	-4.5		-3.4		-2.4		0.0	
COD in-out, mg/d:	446.2		434.1		559.6		716.7	
COD/cm^2-d:	5.50		5.35		6.90		8.84	
CH4out/CH4in:	0.955		0.944		0.949		0.961	
O2/CH4:	0.58		0.75		0.50		0.39	
Time Line, days:	0.51		1.63		4.58		5.55	

	Month	Day	Month	Day	Month	Day	Month	Day
Date:	8	25	8	27	8	28	8	29
Time:	2300		2300		1200		1400	
	In	Out	In	Out	In	Out	In	Out
Oxygen Volume:	79.02	75.82	92.28	91.66	43.65	43.50	47.13	46.45
Methane Volume:	115.23	112.57	103.44	104.47	52.12	51.42	49.49	48.92
Nitrogen Volume:	2.24	4.13	3.52	5.28	1.45	2.98	1.41	2.47
Carb Diox Volume:	0.00	0.00	0.00	0.75	0.00	0.00	0.00	0.27
Total Volume:	196.49	192.52	199.24	202.16	97.22	97.90	98.03	98.11
% Oxygen:	40.22	39.38	46.32	45.34	44.90	44.43	48.08	47.34
% Methane:	58.64	58.47	51.92	51.68	53.61	52.52	50.48	49.86
% Nitrogen:	1.14	2.15	1.77	2.61	1.49	3.04	1.44	2.52
% CO2:	0.00	0.00	0.00	0.37	0.00	0.00	0.00	0.28
Temp, K:								
Gas Flow, ml/min:	10.26	10.26	9.52	9.52	9.73	9.73	9.61	9.61
Gas Press, in H2O:	14.90	4.50	14.50	4.50	12.20	4.50	11.54	4.50
Obs Vol Change, ml								
Act Vol Change, ml								
Oxygen, ml/d:	6159	5883	6576	6284	6479	6294	6842	6624
Methane, ml/d:	8982	8734	7371	7163	7737	7441	7184	6976
Nitrogen, ml/d:	175	320	251	362	215	431	205	352
CO2, ml/d:	0	0	0	51	0	0	0	39
Oxygen, mmol/d:	247.7	236.6	264.5	252.8	260.6	253.2	275.2	266.4
Methane, mmol/d:	361.3	351.3	296.5	288.1	311.2	299.3	289.0	280.6
Nitrogen, mmol/d:	7.0	12.9	10.1	14.6	8.7	17.3	8.2	14.2
CO2, mmol/d:	0.0	0.0	0.0	2.1	0.0	0.0	0.0	1.5
COD, mg/d:	15193.4	14912.5	10510.9	10349.7	11576.5	11052.0	9688.3	9433.0
O2 in-out, mmol/d:	11.1		11.7		7.4		8.8	
CH4 in-out, mmol/d	9.9		8.4		11.9		8.4	
N2 in-out, mmol/d:	-5.9		-4.5		-8.7		-5.9	
CO2 in-out, mmol/d	0.0		-2.1		0.0		-1.5	
COD in-out, mg/d:	280.8		161.2		524.5		255.2	
COD/cm^2-d:	3.46		1.99		6.47		3.15	
CH4out/CH4in:	0.972		0.972		0.962		0.971	
O2/CH4:	1.12		1.40		0.62		1.05	
Time Line, days:	5.96		7.96		8.50		9.58	

	Month	Day	Month	Day	Month	Day	Month	Day
Date:	8	30	8	30	8	31	9	1
Time:	1100		2200		1600		1200	
	In	Out	In	Out	In	Out	In	Out
Oxygen Volume:	42.81	42.84	40.46	40.94	40.34	43.13	23.73	23.73
Methane Volume:	47.66	47.31	49.10	50.08	47.50	50.56	27.54	27.26
Nitrogen Volume:	1.43	2.49	1.21	1.73	0.98	2.33	0.46	0.46
Carb Diox Volume:	0.00	0.27	0.00	0.29	0.00	0.51	0.00	0.19
Total Volume:	91.90	92.91	90.77	93.04	88.82	96.53	51.73	51.64
% Oxygen:	46.58	46.11	44.57	44.00	45.42	44.68	45.87	45.95
% Methane:	51.86	50.92	54.09	53.83	53.48	52.38	53.24	52.79
% Nitrogen:	1.56	2.68	1.33	1.86	1.10	2.41	0.89	0.89
% CO2:	0.00	0.29	0.00	0.31	0.00	0.53	0.00	0.37
Temp, K:								
Gas Flow, ml/min:	9.43	9.43	9.69	9.69	8.90	8.90	8.93	8.93
Gas Press, in H2O:	11.96	4.50	12.00	4.50	11.60	4.50	11.30	4.50
Obs Vol Change, ml								
Act Vol Change, ml								
Oxygen, ml/d:	6512	6331	6403	6208	5987	5790	6063	5975
Methane, ml/d:	7249	6991	7771	7594	7049	6787	7036	6863
Nitrogen, ml/d:	218	368	191	262	145	313	118	116
CO2, ml/d:	0	40	0	44	0	68	0	48
Oxygen, mmol/d:	261.9	254.6	257.6	249.7	240.8	232.9	243.9	240.3
Methane, mmol/d:	291.6	281.2	312.6	305.4	283.5	273.0	283.0	276.1
Nitrogen, mmol/d:	8.7	14.8	7.7	10.6	5.8	12.6	4.7	4.7
CO2, mmol/d:	0.0	1.6	0.0	1.8	0.0	2.8	0.0	1.9
COD, mg/d:	10280.3	9848.6	11761.7	11558.1	10441.0	10019.4	10309.3	9977.9
O2 in-out, mmol/d:	7.3		7.9		7.9		3.5	
CH4 in-out, mmol/d	10.4		7.1		10.6		7.0	
N2 in-out, mmol/d:	-6.1		-2.8		-6.7		0.1	
CO2 in-out, mmol/d	-1.6		-1.8		-2.8		-1.9	
COD in-out, mg/d:	431.7		203.6		421.6		331.5	
COD/cm^2-d:	5.32		2.51		5.20		4.09	
CH4out/CH4in:	0.964		0.977		0.963		0.975	
O2/CH4:	0.70		1.11		0.75		0.51	
Time Line, days:	10.46		10.92		11.67		12.50	

	Month	Day	Month	Day	Month	Day	Month	Day
Date:	9	2	9	2	9	3	9	3
Time:	100		1400		130		1300	
	In	Out	In	Out	In	Out	In	Out
Oxygen Volume:	30.18	32.22	23.94	21.74	30.15	29.76	44.78	29.55
Methane Volume:	63.14	54.72	76.14	76.57	67.05	63.78	52.10	64.94
Nitrogen Volume:	2.04	3.31	1.39	2.88	1.42	2.23	1.29	1.49
Carb Diox Volume:	0.00	0.60	0.00	0.88	0.00	0.79	0.00	0.88
Total Volume:	95.36	90.85	101.47	102.07	98.62	96.56	98.17	96.86
% Oxygen:	31.65	35.47	23.59	21.30	30.57	30.82	45.61	30.51
% Methane:	66.21	60.23	75.04	75.02	67.99	66.05	53.07	67.05
% Nitrogen:	2.14	3.64	1.37	2.82	1.44	2.31	1.31	1.54
% CO2:	0.00	0.66	0.00	0.86	0.00	0.82	0.00	0.91
Temp, K:								
Gas Flow, ml/min:	2.48	1.92	2.25	2.02	2.28	2.12	2.70	2.48
Gas Press, in H2O:	14.20	4.50	12.20	4.50	12.30	4.50	15.08	4.50
Obs Vol Change, ml								
Act Vol Change, ml								
Oxygen, ml/d:	1170	991	787	626	1034	951	1839	1102
Methane, ml/d:	2447	1684	2504	2206	2300	2039	2140	2421
Nitrogen, ml/d:	79	102	46	83	49	71	53	56
CO2, ml/d:	0	18	0	25	0	25	0	33
Oxygen, mmol/d:	47.0	39.9	31.7	25.2	41.6	38.3	74.0	44.3
Methane, mmol/d:	98.4	67.7	100.7	88.7	92.5	82.0	86.1	97.4
Nitrogen, mmol/d:	3.2	4.1	1.8	3.3	2.0	2.9	2.1	2.2
CO2, mmol/d:	0.0	0.7	0.0	1.0	0.0	1.0	0.0	1.3
COD, mg/d:	4794.0	3058.2	5432.8	4873.2	4589.0	4023.8	3141.3	4813.9
O2 in-out, mmol/d:	7.2		6.5		3.3		29.7	
CH4 in-out, mmol/d	30.7		12.0		10.5		-11.3	
N2 in-out, mmol/d:	-0.9		-1.5		-0.9		-0.1	
CO2 in-out, mmol/d	-0.7		-1.0		-1.0		-1.3	
COD in-out, mg/d:	1735.7		559.6		565.1		-1672.6	
COD/cm^2-d:	21.40		6.90		6.97		-20.62	
CH4out/CH4in:	0.688		0.881		0.887		1.131	
O2/CH4:	0.23		0.54		0.32		-2.63	
Time Line, days:	13.04		13.58		14.05		14.54	

	Month	Day	Month	Day	Month	Day	
Date:	9	4	9	4	9	6	
Time:	300		1100		100		
	In	Out	In	Out	In	Out	Head
Oxygen Volume:	42.84	44.00	35.27	35.56	39.51	42.14	1.93
Methane Volume:	51.33	46.66	62.40	61.46	54.69	50.00	0.15
Nitrogen Volume:	1.10	1.65	1.10	1.96	0.82	4.00	95.34
Carb Diox Volume:	0.00	1.04	0.00	1.08	0.00	1.20	0.6
Total Volume:	95.27	93.35	98.77	100.06	95.02	97.34	98.02
% Oxygen:	44.97	47.13	35.71	35.54	41.58	43.29	1.97
% Methane:	53.88	49.98	63.18	61.42	57.56	51.37	0.15
% Nitrogen:	1.15	1.77	1.11	1.96	0.86	4.11	97.27
% CO2:	0.00	1.11	0.00	1.08	0.00	1.23	0.61
Temp, K:							305
Gas Flow, ml/min:	2.34	2.25	2.36	1.94	4.95	4.31	
Gas Press, in H2O:	15.08	4.50	12.38	4.50	10.12	4.50	
Obs Vol Change, ml							0
Act Vol Change, ml							0
Oxygen, ml/d:	1571	1544	1250	1004	3038	2717	
Methane, ml/d:	1883	1637	2212	1735	4205	3223	
Nitrogen, ml/d:	40	58	39	55	63	258	
CO2, ml/d:	0	36	0	30	0	77	
Oxygen, mmol/d:	63.2	62.1	50.3	40.4	136.5	122.1	0.0
Methane, mmol/d:	75.7	65.9	89.0	69.8	188.9	144.8	0.0
Nitrogen, mmol/d:	1.6	2.3	1.6	2.2	2.8	11.6	0.0
CO2, mmol/d:	0.0	1.5	0.0	1.2	0.0	3.5	0.0
COD, mg/d:	2824.2	2227.7	4085.6	3174.1	7724.6	5363.7	
O2 in-out, mmol/d:	1.1		9.9		14.4		
CH4 in-out, mmol/d	9.9		19.2		44.1		
N2 in-out, mmol/d:	-0.7		-0.7		-8.8		
CO2 in-out, mmol/d	-1.5		-1.2		-3.5		
COD in-out, mg/d:	596.5		911.6		2360.9		
COD/cm^2-d:	7.36		11.24		29.11		
CH4out/CH4in:	0.870		0.784		0.767		
O2/CH4:	0.11		0.52		0.33		
Time Line, days:	15.13		15.46		17.04		

	Month	Day		Month	Day	
Date:	9	6		9	6	
Time:	1400			1900		
	In	Out	Head	In	Out	Head
Oxygen Volume:	27.01	19.73	1.52	41.05	36.80	1.29
Methane Volume:	72.17	76.45	0.28	54.79	57.13	0.83
Nitrogen Volume:	1.26	1.45	95.99	1.16	1.55	92.71
Carb Diox Volume:	0.00	0.39	0.74	0.00	0.54	0.79
Total Volume:	100.44	98.02	98.53	97.00	96.02	95.62
% Oxygen:	26.89	20.13	1.54	42.32	38.33	1.35
% Methane:	71.85	77.99	0.28	56.48	59.50	0.87
% Nitrogen:	1.25	1.48	97.42	1.20	1.61	96.96
% CO2:	0.00	0.40	0.75	0.00	0.56	0.83
Temp, K:			305			305
Gas Flow, ml/min:	5.04	4.94		5.61	5.52	
Gas Press, in H2O:	12.00	4.50		12.00	4.50	
Obs Vol Change, ml			0.98			-1.18
Act Vol Change, ml			0.98			-1.18
Oxygen, ml/d:	2009	1448	19.6	3520	3080	23.3
Methane, ml/d:	5369	5610	-6.1	4698	4782	-70.0
Nitrogen, ml/d:	94	106	-9.0	99	130	61.3
CO2, ml/d:	0	29	-6.4	0	45	-9.0
Oxygen, mmol/d:	90.3	65.1	0.9	158.2	138.4	1.0
Methane, mmol/d:	241.3	252.1	-0.3	211.1	214.9	-3.1
Nitrogen, mmol/d:	4.2	4.8	-0.4	4.5	5.8	2.8
CO2, mmol/d:	0.0	1.3	-0.3	0.0	2.0	-0.4
COD, mg/d:	12551.1	14051.4	-45.7	8449.3	9323.0	
O2 in-out, mmol/d:	24.4			18.7		
CH4 in-out, mmol/d	-10.6			-0.6		
N2 in-out, mmol/d:	-0.2			-4.1		
CO2 in-out, mmol/d	-1.0			-1.6		
COD in-out, mg/d:	-1454.6			-873.7		
COD/cm^2-d:	-17.94			-10.77		
CH4out/CH4in:	1.045			1.018		
O2/CH4:	-2.31			-29.66		
Time Line, days:	17.58			17.79		

	Month	Day		Month	Day	
Date:	9	7		9	7	
Time:	600			1700		
	In	Out	Head	In	Out	Head
Oxygen Volume:	10.98	15.03	4.99	13.33	10.30	3.7
Methane Volume:	86.29	79.92	0.51	84.38	84.12	0.76
Nitrogen Volume:	1.30	1.65	91.52	1.06	1.33	90.32
Carb Diox Volume:	0.00	0.44	0.62	0.00	0.37	0.75
Total Volume:	98.57	97.04	97.64	98.77	96.12	95.53
% Oxygen:	11.14	15.49	5.11	13.50	10.72	3.87
% Methane:	87.54	82.36	0.52	85.43	87.52	0.80
% Nitrogen:	1.32	1.70	93.73	1.07	1.38	94.55
% CO2:	0.00	0.45	0.63	0.00	0.38	0.79
Temp, K:			305			304.9
Gas Flow, ml/min:	4.16	3.16		4.06	2.48	
Gas Press, in H2O:	9.38	4.50		4.50	4.50	
Obs Vol Change, ml			-2.15			-0.39
Act Vol Change, ml			-2.15			0.43
Oxygen, ml/d:	683	713	-204.9	798	387	67.5
Methane, ml/d:	5365	3789	18.9	5050	3160	-14.9
Nitrogen, ml/d:	81	78	180.3	63	50	-45.3
CO2, ml/d:	0	21	10.5	0	14	-8.2
Oxygen, mmol/d:	30.7	32.0	-9.2	35.8	17.4	3.0
Methane, mmol/d:	241.1	170.3	0.8	226.9	142.0	-0.7
Nitrogen, mmol/d:	3.6	3.5	8.1	2.8	2.2	-2.0
CO2, mmol/d:	0.0	0.9	0.5	0.0	0.6	-0.4
COD, mg/d:	14448.1	9872.7	349.0	13371.9	8528.8	
O2 in-out, mmol/d:	7.9			15.4		
CH4 in-out, mmol/d	70.0			85.6		
N2 in-out, mmol/d:	-8.0			2.6		
CO2 in-out, mmol/d	-1.4			-0.3		
COD in-out, mg/d:	4226.5			4843.1		
COD/cm^2-d:	52.11			59.72		
CH4out/CH4in:	0.706			0.626		
O2/CH4:	0.11			0.18		
Time Line, days:	18.25			18.71		

	Month	Day		Month	Day	
Date:	9	8		9	8	
Time:	430			900		
	In	Out	Head	In	Out	Head
Oxygen Volume:	46.46	42.69	2.71	47.05	46.08	2.73
Methane Volume:	51.55	55.23	0.98	49.28	48.69	0.83
Nitrogen Volume:	0.92	1.67	94.8	1.56	1.56	96.43
Carb Diox Volume:	0.00	0.31	0.89	0.00	0.35	0.88
Total Volume:	98.93	99.90	99.38	97.89	96.68	100.87
% Oxygen:	46.96	42.73	2.73	48.06	47.66	2.71
% Methane:	52.11	55.29	0.99	50.34	50.36	0.82
% Nitrogen:	0.93	1.67	95.39	1.59	1.61	95.60
% CO2:	0.00	0.31	0.90	0.00	0.36	0.87
Temp, K:			300			300.8
Gas Flow, ml/min:	8.50	8.32		8.43	8.20	
Gas Press, in H2O:	15.92	4.50		15.75	4.50	
Obs Vol Change, ml			-1.17			0.98
Act Vol Change, ml			39.66			-5.67
Oxygen, ml/d:	5973	5176	58.6	6061	5690	3.4
Methane, ml/d:	6628	6697	-10.9	6348	6013	21.1
Nitrogen, ml/d:	118	202	-125.2	201	193	1.3
CO2, ml/d:	0	38	-6.6	0	43	3.2
Oxygen, mmol/d:	264.0	228.8	2.6	268.6	252.2	0.2
Methane, mmol/d:	292.9	296.0	-0.5	281.3	266.5	0.9
Nitrogen, mmol/d:	5.2	9.0	-5.5	8.9	8.5	0.1
CO2, mmol/d:	0.0	1.7	-0.3	0.0	1.9	0.1
COD, mg/d:	10299.8	11622.9	-113.8	9409.7	8984.0	
O2 in-out, mmol/d:	32.6			16.3		
CH4 in-out, mmol/d	-2.6			13.9		
N2 in-out, mmol/d:	1.8			0.3		
CO2 in-out, mmol/d	-1.4			-2.1		
COD in-out, mg/d:	-1209.3			425.7		
COD/cm^2-d:	-14.91			5.25		
CH4out/CH4in:	1.010			0.947		
O2/CH4:	-12.65			1.17		
Time Line, days:	19.18			19.38		

	Month	Day		Month	Day	
Date:	9	8		9	8	
Time:	1830			2400		
	In	Out	Head	In	Out	Head
Oxygen Volume:	43.45	43.90	2.59	43.40	43.16	3.47
Methane Volume:	51.58	51.33	1.22	51.68	51.20	0.84
Nitrogen Volume:	1.17	1.34	94.94	0.89	1.21	89.25
Carb Diox Volume:	0.00	0.45	0.91	0.00	0.35	0.84
Total Volume:	96.20	97.02	99.66	95.97	95.92	94.40
% Oxygen:	45.17	45.25	2.60	45.22	45.00	3.68
% Methane:	53.62	52.91	1.22	53.85	53.38	0.89
% Nitrogen:	1.22	1.38	95.26	0.93	1.26	94.54
% CO2:	0.00	0.46	0.91	0.00	0.36	0.89
Temp, K:			301.1			301.8
Gas Flow, ml/min:	8.73	8.71		9.02	8.86	
Gas Press, in H2O:	16.29	4.50		16.46	4.50	
Obs Vol Change, ml			-0.39			0.78
Act Vol Change, ml			-2.88			-5.02
Oxygen, ml/d:	5905	5738	7.1	6112	5804	-112.6
Methane, ml/d:	7010	6709	-25.8	7278	6885	35.4
Nitrogen, ml/d:	159	175	28.7	125	163	95.7
CO2, ml/d:	0	59	-2.6	0	47	2.6
Oxygen, mmol/d:	262.0	254.6	0.3	271.8	258.1	-5.0
Methane, mmol/d:	311.0	297.6	-1.1	323.6	306.2	1.6
Nitrogen, mmol/d:	7.1	7.8	1.3	5.6	7.2	4.3
CO2, mmol/d:	0.0	2.6	-0.1	0.0	2.1	0.1
COD, mg/d:	11520.5	10903.1	-83.4	12014.3	11336.0	
O2 in-out, mmol/d:	7.1			18.7		
CH4 in-out, mmol/d	14.5			15.9		
N2 in-out, mmol/d:	-2.0			-5.9		
CO2 in-out, mmol/d	-2.5			-2.2		
COD in-out, mg/d:	700.8			678.4		
COD/cm^2-d:	8.64			8.36		
CH4out/CH4in:	0.957			0.946		
O2/CH4:	0.49			1.18		
Time Line, days:	19.76			20.00		

	Month	Day		Month	Day	
Date:	9	9		9	9	
Time:	830			1230		
	In	Out	Head	In	Out	Head
Oxygen Volume:	47.25	46.16	2.05	44.68	44.19	2.28
Methane Volume:	51.53	50.58	0.83	52.34	51.15	0.72
Nitrogen Volume:	1.29	1.54	94.87	1.30	1.63	95.01
Carb Diox Volume:	0.00	0.40	0.9	0.00	0.42	0.97
Total Volume:	100.07	98.68	98.65	98.32	97.39	98.98
% Oxygen:	47.22	46.78	2.08	45.44	45.37	2.30
% Methane:	51.49	51.26	0.84	53.23	52.52	0.73
% Nitrogen:	1.29	1.56	96.17	1.32	1.67	95.99
% CO2:	0.00	0.41	0.91	0.00	0.43	0.98
Temp, K:			301.8			302.1
Gas Flow, ml/min:	8.60	8.53		8.69	8.59	
Gas Press, in H2O:	16.54	4.50		16.21	4.50	
Obs Vol Change, ml			-1.8			-2.03
Act Vol Change, ml			-1.80			-4.51
Oxygen, ml/d:	6085	5809	115.6	5913	5675	-33.2
Methane, ml/d:	6636	6366	3.5	6927	6568	17.3
Nitrogen, ml/d:	166	194	-112.4	172	209	52.9
CO2, ml/d:	0	50	-1.6	0	54	-9.9
Oxygen, mmol/d:	270.6	258.3	5.1	263.2	252.6	-1.5
Methane, mmol/d:	295.1	283.1	0.2	308.3	292.4	0.8
Nitrogen, mmol/d:	7.4	8.6	-5.0	7.7	9.3	2.4
CO2, mmol/d:	0.0	2.2	-0.1	0.0	2.4	-0.4
COD, mg/d:	10227.2	9849.2	-154.4	11310.2	10628.7	
O2 in-out, mmol/d:	7.1			12.1		
CH4 in-out, mmol/d	11.9			15.2		
N2 in-out, mmol/d:	3.8			-4.0		
CO2 in-out, mmol/d	-2.2			-2.0		
COD in-out, mg/d:	532.4			681.5		
COD/cm^2-d:	6.56			8.40		
CH4out/CH4in:	0.959			0.948		
O2/CH4:	0.60			0.80		
Time Line, days:	20.35			20.51		

	Month	Day		Month	Day	
Date:	9	9		9	9	
Time:	1630			2230		
	In	Out	Head	In	Out	Head
Oxygen Volume:	42.96	42.71	2.6	43.61	42.43	2.68
Methane Volume:	52.60	52.24	0.72	52.89	51.76	0.76
Nitrogen Volume:	1.25	1.33	92.33	1.21	1.39	95.07
Carb Diox Volume:	0.00	0.41	0.92	0.00	0.38	0.96
Total Volume:	96.81	96.69	96.57	97.71	95.96	99.47
% Oxygen:	44.38	44.17	2.69	44.63	44.22	2.69
% Methane:	54.33	54.03	0.75	54.13	53.94	0.76
% Nitrogen:	1.29	1.38	95.61	1.24	1.45	95.58
% CO2:	0.00	0.42	0.95	0.00	0.40	0.97
Temp, K:			302.1			302.2
Gas Flow, ml/min:	8.94	8.93		9.12	9.07	
Gas Press, in H2O:	16.54	4.50		16.50	4.50	
Obs Vol Change, ml			-2.03			-3.05
Act Vol Change, ml			-2.03			-3.88
Oxygen, ml/d:	5945	5743	-58.0	6099	5839	0.2
Methane, ml/d:	7279	7024	-2.6	7397	7123	-1.7
Nitrogen, ml/d:	173	179	68.6	169	191	18.1
CO2, ml/d:	0	55	4.2	0	52	-1.1
Oxygen, mmol/d:	264.6	255.6	-2.6	271.6	260.0	0.0
Methane, mmol/d:	324.0	312.7	-0.1	329.4	317.1	-0.1
Nitrogen, mmol/d:	7.7	8.0	3.1	7.5	8.5	0.8
CO2, mmol/d:	0.0	2.5	0.2	0.0	2.3	0.0
COD, mg/d:	12267.8	11830.4	75.1	12388.5	11977.9	
O2 in-out, mmol/d:	11.6			11.6		
CH4 in-out, mmol/d	11.4			12.3		
N2 in-out, mmol/d:	-3.3			-1.8		
CO2 in-out, mmol/d	-2.6			-2.3		
COD in-out, mg/d:	362.4			410.6		
COD/cm^2-d:	4.47			5.06		
CH4out/CH4in:	0.965			0.963		
O2/CH4:	1.01			0.94		
Time Line, days:	20.68			20.93		

	Month	Day		Month	Day	
Date:	9	10		9	10	
Time:	330			1200		
	In	Out	Head	In	Out	Head
Oxygen Volume:	42.71	42.26	2.79	46.59	45.85	2.7
Methane Volume:	52.33	52.07	0.77	52.03	51.30	0.73
Nitrogen Volume:	1.54	1.71	91.71	0.99	1.65	91.44
Carb Diox Volume:	0.00	0.40	0.99	0.00	0.35	1.1
Total Volume:	96.58	96.44	96.26	99.61	99.15	95.97
% Oxygen:	44.22	43.82	2.90	46.77	46.24	2.81
% Methane:	54.18	53.99	0.80	52.23	51.74	0.76
% Nitrogen:	1.59	1.77	95.27	0.99	1.66	95.28
% CO2:	0.00	0.41	1.03	0.00	0.35	1.15
Temp, K:			302.2			302.3
Gas Flow, ml/min:	9.31	9.31		9.19	9.06	
Gas Press, in H2O:	17.04	4.50		16.79	4.50	
Obs Vol Change, ml			-2.54			-1.38
Act Vol Change, ml			-2.54			-2.21
Oxygen, ml/d:	6177	5940	-24.1	6445	6100	6.0
Methane, ml/d:	7568	7318	-4.2	7198	6825	2.8
Nitrogen, ml/d:	223	240	48.0	137	220	5.3
CO2, ml/d:	0	56	-7.5	0	47	-8.0
Oxygen, mmol/d:	275.0	264.5	-1.1	287.1	271.7	0.3
Methane, mmol/d:	337.0	325.9	-0.2	320.6	304.0	0.1
Nitrogen, mmol/d:	9.9	10.7	2.1	6.1	9.8	0.2
CO2, mmol/d:	0.0	2.5	-0.3	0.0	2.1	-0.4
COD, mg/d:	12765.6	12391.9	22.4	11331.3	10760.7	
O2 in-out, mmol/d:	11.6			15.1		
CH4 in-out, mmol/d	11.3			16.5		
N2 in-out, mmol/d:	-2.9			-3.9		
CO2 in-out, mmol/d	-2.2			-1.7		
COD in-out, mg/d:	351.3			570.6		
COD/cm^2-d:	4.33			7.04		
CH4out/CH4in:	0.967			0.948		
O2/CH4:	1.03			0.92		
Time Line, days:	21.14			21.50		

	Month	Day	Month	Day	Month	Day	
Date:	9	11	9	12	9	17	
Time:	1300		1300		1300		
	In	Out	In	Out	In	Out	Head
Oxygen Volume:	43.82	42.57	41.90	42.25	44.31	44.31	2.51
Methane Volume:	51.81	50.65	52.16	52.84	51.27	51.27	0
Nitrogen Volume:	1.57	1.89	1.40	1.40	1.29	1.44	92.25
Carb Diox Volume:	0.00	0.33	0.00	0.46	0.00	0.32	0.37
Total Volume:	97.20	95.44	95.46	96.95	96.87	97.34	95.13
% Oxygen:	45.08	44.60	43.89	43.58	45.74	45.52	2.64
% Methane:	53.30	53.07	54.64	54.50	52.93	52.67	0.00
% Nitrogen:	1.62	1.98	1.47	1.44	1.33	1.48	96.97
% CO2:	0.00	0.35	0.00	0.47	0.00	0.33	0.39
Temp, K:							302.6
Gas Flow, ml/min:	9.37	9.34	9.57	9.52	9.00	8.96	
Gas Press, in H2O:	16.71	4.50	17.21	4.50	17.12	4.50	
Obs Vol Change, ml							0
Act Vol Change, ml							0.00
Oxygen, ml/d:	6333	6065	6305	6040	6178	5938	35.9
Methane, ml/d:	7487	7217	7848	7554	7148	6871	47.7
Nitrogen, ml/d:	227	269	211	200	180	193	-83.7
CO2, ml/d:	0	47	0	66	0	43	0.1
Oxygen, mmol/d:	254.7	244.0	253.6	243.0	275.4	264.8	1.6
Methane, mmol/d:	301.2	290.3	315.7	303.9	318.7	306.3	2.1
Nitrogen, mmol/d:	9.1	10.8	8.5	8.1	8.0	8.6	-3.7
CO2, mmol/d:	0.0	1.9	0.0	2.6	0.0	1.9	0.0
COD, mg/d:	11123.5	10770.6	12089.1	11672.0	11582.3	11133.5	
O2 in-out, mmol/d:	10.8		10.6		9.1		
CH4 in-out, mmol/d	10.9		11.8		10.2		
N2 in-out, mmol/d:	-1.7		0.4		3.1		
CO2 in-out, mmol/d	-1.9		-2.6		-1.9		
COD in-out, mg/d:	352.9		417.1		448.8		
COD/cm^2-d:	4.35		5.14		5.53		
CH4out/CH4in:	0.964		0.963		0.961		
O2/CH4:	0.99		0.90		0.89		
Time Line, days:	22.54		23.54		28.54		

	Month	Day		Month	Day	
Date:	9	19		9	20	
Time:	1400			2330		
	In	Out	Head	In	Out	Head
Oxygen Volume:	42.26	41.81	8.62	53.66	52.69	8.91
Methane Volume:	54.65	54.35	0.2	46.57	46.76	0.37
Nitrogen Volume:	0.61	0.81	86.87	1.58	1.85	93.19
Carb Diox Volume:	0.00	0.32	0.23	0.00	0.33	0.14
Total Volume:	97.52	97.29	95.92	101.81	101.63	102.61
% Oxygen:	43.33	42.97	8.99	52.71	51.84	8.68
% Methane:	56.04	55.86	0.21	45.74	46.01	0.36
% Nitrogen:	0.63	0.83	90.57	1.55	1.82	90.82
% CO2:	0.00	0.33	0.24	0.00	0.32	0.14
Temp, K:			302.3			302.2
Gas Flow, ml/min:	9.60	9.34		11.34	10.87	
Gas Press, in H2O:	9.12	4.50		11.21	4.50	
Obs Vol Change, ml			0			0
Act Vol Change, ml			2.48			0.83
Oxygen, ml/d:	6125	5844	-77.8	8844	8205	5.4
Methane, ml/d:	7921	7597	-2.6	7675	7282	-2.7
Nitrogen, ml/d:	88	113	77.4	260	288	-5.1
CO2, ml/d:	0	45	1.8	0	51	1.9
Oxygen, mmol/d:	272.8	260.3	-3.5	393.8	365.3	0.2
Methane, mmol/d:	352.8	338.3	-0.1	341.7	324.2	-0.1
Nitrogen, mmol/d:	3.9	5.0	3.4	11.6	12.8	-0.2
CO2, mmol/d:	0.0	2.0	0.1	0.0	2.3	0.1
COD, mg/d:	13848.4	13325.3	103.7	9270.9	9059.0	
O2 in-out, mmol/d:	16.0			28.2		
CH4 in-out, mmol/d	14.5			17.7		
N2 in-out, mmol/d:	-4.6			-1.0		
CO2 in-out, mmol/d	-2.1			-2.4		
COD in-out, mg/d:	419.4			211.9		
COD/cm^2-d:	5.17			2.61		
CH4out/CH4in:	0.959			0.949		
O2/CH4:	1.10			1.60		
Time Line, days:	30.58			31.97		

	Month	Day		Month	Day	
Date:	9	21		9	22	
Time:	1330			20		
	In	Out	Head	In	Out	Head
Oxygen Volume:	51.35	50.61	13.83	48.37	50.38	17.75
Methane Volume:	47.52	46.82	0.35	46.68	48.52	0.18
Nitrogen Volume:	1.55	1.75	86.45	1.12	1.38	80.09
Carb Diox Volume:	0.00	0.66	0.28	0.00	0.63	0.1
Total Volume:	100.42	99.84	100.91	96.17	100.91	98.12
% Oxygen:	51.14	50.69	13.71	50.30	49.93	18.09
% Methane:	47.32	46.90	0.35	48.54	48.08	0.18
% Nitrogen:	1.54	1.75	85.67	1.16	1.37	81.62
% CO2:	0.00	0.66	0.28	0.00	0.62	0.10
Temp, K:			302.5			302.6
Gas Flow, ml/min:	11.60	10.85		11.21	10.76	
Gas Press, in H2O:	11.29	4.50		11.21	4.50	
Obs Vol Change, ml			0			0
Act Vol Change, ml			-2.48			-0.83
Oxygen, ml/d:	8779	8008	-214.6	8343	7821	-241.0
Methane, ml/d:	8124	7408	0.6	8051	7532	9.0
Nitrogen, ml/d:	265	277	224.3	193	214	224.2
CO2, ml/d:	0	104	-6.0	0	98	9.7
Oxygen, mmol/d:	391.3	356.9	-9.6	372.0	348.7	-10.7
Methane, mmol/d:	362.1	330.2	0.0	359.0	335.8	0.4
Nitrogen, mmol/d:	11.8	12.3	10.0	8.6	9.6	10.0
CO2, mmol/d:	0.0	4.7	-0.3	0.0	4.4	0.4
COD, mg/d:	10652.6	9710.1	307.8	11070.8	10334.5	
O2 in-out, mmol/d:	43.9			34.0		
CH4 in-out, mmol/d	31.9			22.7		
N2 in-out, mmol/d:	-10.5			-10.9		
CO2 in-out, mmol/d	-4.4			-4.8		
COD in-out, mg/d:	634.7			736.2		
COD/cm^2-d:	7.83			9.08		
CH4out/CH4in:	0.912			0.936		
O2/CH4:	1.38			1.50		
Time Line, days:	32.55			33.01		

	Month	Day		Month	Day	
Date:	9	22		9	23	
Time:	1920			1240		
	In	Out	Head	In	Out	Head
Oxygen Volume:	50.85	50.80	12.8	52.46	52.25	12.77
Methane Volume:	48.39	48.30	0.36	44.96	45.21	0.25
Nitrogen Volume:	1.53	1.53	87.47	1.39	2.03	85.82
Carb Diox Volume:	0.00	0.62	0.38	0.00	0.61	0.37
Total Volume:	100.77	101.25	101.01	98.81	100.10	99.21
% Oxygen:	50.46	50.17	12.67	53.09	52.20	12.87
% Methane:	48.02	47.70	0.36	45.50	45.16	0.25
% Nitrogen:	1.52	1.51	86.60	1.41	2.03	86.50
% CO2:	0.00	0.61	0.38	0.00	0.61	0.37
Temp, K:			302.4			301
Gas Flow, ml/min:	10.55	10.38		9.75	9.41	
Gas Press, in H2O:	16.00	4.50		16.00	4.50	
Obs Vol Change, ml			0			0
Act Vol Change, ml			1.65			11.63
Oxygen, ml/d:	7968	7582	170.8	7747	7151	-9.1
Methane, ml/d:	7582	7209	-5.5	6640	6188	3.6
Nitrogen, ml/d:	240	228	-158.8	205	278	-10.8
CO2, ml/d:	0	93	-8.7	0	83	0.1
Oxygen, mmol/d:	355.0	337.8	7.6	343.6	317.1	-0.4
Methane, mmol/d:	337.8	321.2	-0.2	294.5	274.4	0.2
Nitrogen, mmol/d:	10.7	10.2	-7.1	9.1	12.3	-0.5
CO2, mmol/d:	0.0	4.1	-0.4	0.0	3.7	0.0
COD, mg/d:	10260.7	9746.5	-259.2	7850.8	7413.8	
O2 in-out, mmol/d:	9.6			26.8		
CH4 in-out, mmol/d	16.9			19.9		
N2 in-out, mmol/d:	7.6			-2.7		
CO2 in-out, mmol/d	-3.7			-3.7		
COD in-out, mg/d:	773.3			436.9		
COD/cm^2-d:	9.54			5.39		
CH4out/CH4in:	0.951			0.932		
O2/CH4:	0.57			1.35		
Time Line, days:	33.80			34.52		

	Month	Day		Month	Day	
Date:	9	23		9	24	
Time:	2230			1350		
	In	Out	Head	In	Out	Head
Oxygen Volume:	52.77	53.20	13.03	49.66	48.28	3.07
Methane Volume:	45.80	45.97	0.35	47.72	46.71	0.51
Nitrogen Volume:	1.41	1.61	85.44	1.45	1.58	94.29
Carb Diox Volume:	0.00	0.42	0.47	0.00	0.64	0.97
Total Volume:	99.98	101.20	99.29	98.83	97.21	98.84
% Oxygen:	52.78	52.57	13.12	50.25	49.67	3.11
% Methane:	45.81	45.42	0.35	48.28	48.05	0.52
% Nitrogen:	1.41	1.59	86.05	1.47	1.63	95.40
% CO2:	0.00	0.42	0.47	0.00	0.66	0.98
Temp, K:			301			301.6
Gas Flow, ml/min:	11.48	11.25		11.34	11.11	
Gas Press, in H2O:	15.67	4.50		8.83	4.50	
Obs Vol Change, ml			0			0
Act Vol Change, ml			0.00			-4.97
Oxygen, ml/d:	9061	8611	-15.2	8383	8036	395.7
Methane, ml/d:	7865	7441	-6.1	8056	7775	-6.4
Nitrogen, ml/d:	242	261	27.4	245	263	-361.4
CO2, ml/d:	0	68	-6.1	0	107	-20.0
Oxygen, mmol/d:	401.9	381.9	-0.7	372.5	357.1	17.6
Methane, mmol/d:	348.8	330.0	-0.3	358.0	345.5	-0.3
Nitrogen, mmol/d:	10.7	11.6	1.2	10.9	11.7	-16.1
CO2, mmol/d:	0.0	3.0	-0.3	0.0	4.7	-0.9
COD, mg/d:	9462.4	8898.4	4.3	10989.5	10683.7	
O2 in-out, mmol/d:	20.7			-2.1		
CH4 in-out, mmol/d	19.1			12.8		
N2 in-out, mmol/d:	-2.0			15.3		
CO2 in-out, mmol/d	-2.7			-3.8		
COD in-out, mg/d:	559.6			305.9		
COD/cm^2-d:	6.90			3.77		
CH4out/CH4in:	0.946			0.965		
O2/CH4:	1.08			-0.17		
Time Line, days:	34.93			35.56		

	Month	Day		Month	Day	
Date:	9	24		9	25	
Time:	2330			2230		
	In	Out	Head	In	Out	Head
Oxygen Volume:	48.14	47.72	3.4	47.29	47.34	3.94
Methane Volume:	48.54	48.37	0.42	49.16	49.58	0.33
Nitrogen Volume:	1.69	1.75	96	0.68	0.68	92.49
Carb Diox Volume:	0.00	0.51	0.98	0.00	0.59	1.01
Total Volume:	98.37	98.35	100.80	97.13	98.19	97.77
% Oxygen:	48.94	48.52	3.37	48.69	48.21	4.03
% Methane:	49.34	49.18	0.42	50.61	50.49	0.34
% Nitrogen:	1.72	1.78	95.24	0.70	0.69	94.60
% CO2:	0.00	0.52	0.97	0.00	0.60	1.03
Temp, K:			302			301.4
Gas Flow, ml/min:	11.66	11.43		11.41	11.18	
Gas Press, in H2O:	8.83	4.50		9.29	4.50	
Obs Vol Change, ml			0			0
Act Vol Change, ml			-3.31			4.98
Oxygen, ml/d:	8395	8072	-16.1	8182	7849	-17.3
Methane, ml/d:	8465	8182	6.1	8506	8220	2.0
Nitrogen, ml/d:	295	296	17.4	118	113	11.7
CO2, ml/d:	0	86	0.6	0	98	-1.6
Oxygen, mmol/d:	373.5	359.2	-0.7	363.3	348.6	-0.8
Methane, mmol/d:	376.7	364.1	0.3	377.7	365.0	0.1
Nitrogen, mmol/d:	13.1	13.2	0.8	5.2	5.0	0.5
CO2, mmol/d:	0.0	3.8	0.0	0.0	4.3	-0.1
COD, mg/d:	12152.2	11806.7	40.3	12546.7	12209.1	
O2 in-out, mmol/d:	15.1			15.6		
CH4 in-out, mmol/d	12.3			12.6		
N2 in-out, mmol/d:	-0.8			-0.3		
CO2 in-out, mmol/d	-3.9			-4.3		
COD in-out, mg/d:	305.1			337.6		
COD/cm^2-d:	3.76			4.16		
CH4out/CH4in:	0.967			0.966		
O2/CH4:	1.23			1.24		
Time Line, days:	35.97			36.93		

	Month	Day		Month	Day	
Date:	9	26		9	27	
Time:	1950			1820		
	In	Out	Head	In	Out	Head
Oxygen Volume:	49.38	49.08	5.08	50.89	50.05	5.18
Methane Volume:	50.00	49.60	0.48	47.48	46.74	0.44
Nitrogen Volume:	1.73	1.55	95.07	1.65	1.70	94.5
Carb Diox Volume:	0.00	0.62	1.24	0.00	0.60	1.37
Total Volume:	101.11	100.85	101.87	100.02	99.09	101.49
% Oxygen:	48.84	48.67	4.99	50.88	50.51	5.10
% Methane:	49.45	49.18	0.47	47.47	47.17	0.43
% Nitrogen:	1.71	1.54	93.32	1.65	1.72	93.11
% CO2:	0.00	0.61	1.22	0.00	0.61	1.35
Temp, K:			302.5			302.7
Gas Flow, ml/min:	11.26	11.03		11.42	11.19	
Gas Press, in H2O:	9.12	4.50		9.04	4.50	
Obs Vol Change, ml			0			0
Act Vol Change, ml			-9.09			-1.65
Oxygen, ml/d:	8096	7819	-26.6	8553	8230	-3.0
Methane, ml/d:	8198	7902	-3.7	7980	7686	1.0
Nitrogen, ml/d:	284	247	45.7	277	280	7.2
CO2, ml/d:	0	99	-5.1	0	99	-3.5
Oxygen, mmol/d:	360.8	348.5	-1.2	381.5	367.1	-0.1
Methane, mmol/d:	365.4	352.2	-0.2	355.9	342.8	0.0
Nitrogen, mmol/d:	12.6	11.0	2.0	12.4	12.5	0.3
CO2, mmol/d:	0.0	4.4	-0.2	0.0	4.4	-0.2
COD, mg/d:	11837.0	11387.4	27.2	10570.7	10192.1	
O2 in-out, mmol/d:	13.6			14.5		
CH4 in-out, mmol/d	13.4			13.1		
N2 in-out, mmol/d:	-0.4			-0.4		
CO2 in-out, mmol/d	-4.2			-4.2		
COD in-out, mg/d:	422.4			378.5		
COD/cm^2-d:	5.21			4.67		
CH4out/CH4in:	0.964			0.963		
O2/CH4:	1.01			1.11		
Time Line, days:	37.81			38.76		

	Month	Day	
Date:	10	10	
Time:	1200		
	In	Out	Head
Oxygen Volume:	55.87	54.54	4.01
Methane Volume:	45.02	44.33	0.28
Nitrogen Volume:	1.67	1.96	95.06
Carb Diox Volume:	0.00	0.53	2.53
Total Volume:	102.56	101.36	101.88
% Oxygen:	54.48	53.81	3.94
% Methane:	43.90	43.74	0.27
% Nitrogen:	1.63	1.93	93.31
% CO2:	0.00	0.52	2.48
Temp, K:			303.6
Gas Flow, ml/min:	8.99	8.61	
Gas Press, in H2O:	14.92	4.50	
Obs Vol Change, ml			0
Act Vol Change, ml			-7.41
Oxygen, ml/d:	7311	6745	2.3
Methane, ml/d:	5891	5482	0.3
Nitrogen, ml/d:	219	242	0.2
CO2, ml/d:	0	66	-2.2
Oxygen, mmol/d:	327.0	301.7	0.1
Methane, mmol/d:	263.5	245.2	0.0
Nitrogen, mmol/d:	9.8	10.8	0.0
CO2, mmol/d:	0.0	2.9	-0.1
COD, mg/d:	6400.2	6040.1	-2.4
O2 in-out, mmol/d:	25.2		
CH4 in-out, mmol/d	18.3		
N2 in-out, mmol/d:	-1.1		
CO2 in-out, mmol/d	-2.8		
COD in-out, mg/d:	362.5		
COD/cm^2-d:	4.47		
CH4out/CH4in:	0.931		
O2/CH4:	1.38		
Time Line, days:	51.50		

APPENDIX B

Chlorinated Compounds Data Spreadsheet

GC areas and gas flows are measured values. All other values are calculated.

	CH2C12 GC Area			CHC13 GC Area			CC14 GC Area		
t, hr	Liq	Head	Mem Out	Liq	Head	Mem Out	Liq	Head	Mem Out
2	40.37	10.20	5.33	21.58	10.88	3.43	5.94	17.70	4.86
5	37.28	11.14	5.40	21.19	12.32	3.29	3.85	30.03	3.10
9	35.28	11.85	3.20	20.15	12.58	3.08	3.19	33.66	1.28
18	32.97	11.48	2.72	18.85	13.20	3.00	2.53	36.20	1.04
25	30.91	11.05	1.28	17.94	13.20	2.66	2.42	33.57	0.96
37	25.41	9.00	1.04	15.99	11.36	2.08	2.31	28.53	0.80
48	18.80	6.32	0.78	14.75	10.48	2.03	2.20	27.63	0.90
67	12.00	5.28	0.54	13.25	9.52	2.02	1.87	28.17	0.75
85	6.32	3.44	0.14	11.75	9.52	1.36	1.87	30.42	0.98
94	5.10	2.25	0.05	11.25	8.26	1.30	1.76	24.66	0.34
110	3.23	1.65	0.00	10.50	7.95	1.20	1.90	27.09	0.75
119	2.52	1.42	0.00	10.00	7.21	1.02	1.65	25.29	0.46
142	1.04	0.52	0.00	7.88	6.11	0.91	1.65	22.86	0.45
164	0.00	0.18	0.00	6.88	5.85	0.84	1.21	22.36	0.52
186	0.00	0.00	0.00	6.25	4.62	0.66	1.21	22.05	0.45

	CH2C12 Concentration			CHC13 Concentration			CC14 Concentration		
t, hr	Liq	Head	Mem Out	Liq	Head	Mem Out	Liq	Head	Mem Out
2	10.87	0.47	0.27	9.88	0.64	0.20	19.59	3.55	1.00
5	10.04	0.50	0.27	9.70	0.72	0.19	13.17	5.97	0.64
9	9.50	0.53	0.17	9.23	0.74	0.18	11.09	6.68	0.27
18	8.88	0.52	0.15	8.63	0.77	0.17	8.97	7.17	0.22
25	8.33	0.50	0.08	8.22	0.77	0.15	8.61	6.66	0.20
37	6.85	0.42	0.06	7.33	0.66	0.12	8.26	5.68	0.17
48	5.08	0.31	0.05	6.76	0.61	0.11	7.89	5.50	0.19
67	3.25	0.26	0.04	6.07	0.55	0.11	6.80	5.61	0.16
85	1.71	0.18	0.01	5.39	0.55	0.08	6.80	6.05	0.21
94	1.38	0.13	0.00	5.16	0.48	0.07	6.44	4.92	0.07
110	0.88	0.10	0.00	4.81	0.46	0.07	6.90	5.39	0.16
119	0.69	0.08	0.00	4.59	0.42	0.06	6.07	5.04	0.10
142	0.28	0.04	0.00	3.62	0.35	0.05	6.07	4.56	0.10
164	0.00	0.01	0.00	3.16	0.34	0.05	4.57	4.47	0.11
186	0.00	0.00	0.00	2.87	0.27	0.04	4.57	4.41	0.10

********** FITTED DATA **********

	CH2Cl2 Concentration			CHCl3 Concentration			CCl4 Concentration		
t, hr	Liq	Head	Mem Out	Liq	Head	Mem Out	Liq	Head	Mem Out
2	10.58	0.471	0.272	9.83	0.650	0.196	19.12	3.29	0.956
5	10.07	0.505	0.241	9.59	0.713	0.188	13.39	5.96	0.517
9	9.61	0.527	0.203	9.22	0.748	0.176	10.96	6.66	0.290
18	8.96	0.532	0.147	8.53	0.774	0.157	9.10	7.08	0.240
25	8.31	0.500	0.115	8.04	0.762	0.143	8.65	6.49	0.210
37	6.70	0.418	0.073	7.30	0.668	0.126	8.16	6.10	0.180
48	5.21	0.345	0.047	6.74	0.615	0.112	7.72	5.87	0.157
67	3.18	0.238	0.022	5.96	0.553	0.094	7.14	5.53	0.122
85	1.81	0.158	0.010	5.38	0.510	0.081	6.60	5.28	0.105
94	1.32	0.128	0.006	5.10	0.488	0.074	6.41	5.16	0.095
110	0.79	0.087	0.002	4.65	0.451	0.066	6.07	4.99	0.090
119	0.56	0.071	0.001	4.42	0.430	0.062	5.88	4.88	0.085
142	0.22	0.039	0.000	3.89	0.378	0.053	5.43	4.67	0.075
164	0.06	0.016	0.000	3.41	0.331	0.046	5.03	4.45	0.075
186	0.00	0.002	0.000	2.92	0.284	0.038	4.61	4.28	0.075

	CH2Cl2 Mass Present			CHCl3 Mass Present			CCl4 Mass Present		
t, hr	Liq	Head	Total	Liq	Head	Total	Liq	Head	Total
2	19.47	0.99	20.46	18.09	1.37	19.45	35.18	6.91	42.09
5	18.53	1.06	19.59	17.65	1.50	19.14	24.64	12.52	37.15
9	17.68	1.11	18.79	16.96	1.57	18.54	20.17	13.99	34.15
18	16.49	1.12	17.60	15.70	1.63	17.32	16.74	14.87	31.61
25	15.29	1.05	16.34	14.79	1.60	16.39	15.92	13.63	29.55
37	12.33	0.88	13.21	13.43	1.40	14.83	15.01	12.81	27.82
48	9.59	0.72	10.31	12.40	1.29	13.69	14.20	12.33	26.53
67	5.85	0.50	6.35	10.97	1.16	12.13	13.14	11.61	24.75
85	3.33	0.33	3.66	9.90	1.07	10.97	12.14	11.09	23.23
94	2.43	0.27	2.70	9.38	1.02	10.41	11.79	10.84	22.63
110	1.45	0.18	1.64	8.56	0.95	9.50	11.17	10.48	21.65
119	1.03	0.15	1.18	8.13	0.90	9.04	10.82	10.25	21.07
142	0.40	0.08	0.49	7.16	0.79	7.95	9.99	9.81	19.80
164	0.11	0.03	0.14	6.27	0.70	6.97	9.26	9.35	18.60
186	0.00	0.00	0.00	5.37	0.60	5.97	8.48	8.99	17.47

t, hr	Rate of CH2Cl2 Loss Through Membrane Over Time Interval			Rate of CHCl3 Loss Through Membrane Over Time Interval			Rate of CCl4 Loss Throug Membrane Over Time Inter		
	Initial umol/hr	Final umol/hr	Average umol/hr	Initial umol/hr	Final umol/hr	Average umol/hr	Initial umol/hr	Final umol/hr	Average umol/hr
2	0.0000	0.1618	0.0809	0.0000	0.1168	0.0584	0.0000	0.5698	0.2849
5	0.1618	0.1394	0.1506	0.1168	0.1087	0.1128	0.5698	0.2990	0.4344
9	0.1394	0.1173	0.1283	0.1087	0.1017	0.1052	0.2990	0.1676	0.2333
18	0.1173	0.0843	0.1008	0.1017	0.0900	0.0959	0.1676	0.1376	0.1526
25	0.0843	0.0743	0.0793	0.0900	0.0928	0.0914	0.1376	0.1363	0.1370
37	0.0743	0.0472	0.0608	0.0928	0.0820	0.0874	0.1363	0.1172	0.1268
48	0.0472	0.0303	0.0388	0.0820	0.0723	0.0772	0.1172	0.1014	0.1093
67	0.0303	0.0134	0.0219	0.0723	0.0585	0.0654	0.1014	0.0760	0.0887
85	0.0134	0.0055	0.0094	0.0585	0.0457	0.0521	0.0760	0.0593	0.0676
94	0.0055	0.0041	0.0048	0.0457	0.0500	0.0478	0.0593	0.0641	0.0617
110	0.0041	0.0013	0.0027	0.0500	0.0440	0.0470	0.0641	0.0600	0.0621
119	0.0013	0.0003	0.0008	0.0440	0.0425	0.0433	0.0600	0.0583	0.0591
142	0.0003	0.0000	0.0002	0.0425	0.0356	0.0390	0.0583	0.0503	0.0543
164	0.0000	0.0000	0.0000	0.0356	0.0305	0.0330	0.0503	0.0497	0.0500
186	0.0000	0.0000	0.0000	0.0305	0.0255	0.0280	0.0497	0.0504	0.0500

t, hr	Mass Lost Through Membrane Over Time Interval Delta t			Mass Degraded Over Time Interval Delta t			Rate of Mass Degradation Over Time Interval Delta		
	CH2Cl2 umol	CHCl3 umol	CCl4 umol	CH2Cl2 umol	CHCl3 umol	CCl4 umol	CH2Cl2 umol	CHCl3 umol	CCl4 umol
2	0.1618	0.1168	0.5698	0.000	0.000	0.000	0.0000	0.0000	0.0000
5	0.4518	0.3383	1.3032	0.415	-0.029	3.633	0.1384	-0.0097	1.2110
9	0.5134	0.4208	0.9331	0.287	0.186	2.068	0.0717	0.0466	0.5170
18	0.9071	0.8627	1.3732	0.278	0.352	1.167	0.0309	0.0391	0.1297
25	0.5551	0.6399	0.9587	0.708	0.287	1.108	0.1012	0.0410	0.1583
37	0.7292	1.0492	1.5211	2.405	0.510	0.199	0.2004	0.0425	0.0166
48	0.4265	0.8489	1.2021	2.468	0.293	0.091	0.2244	0.0266	0.0082
67	0.4155	1.2433	1.6850	3.544	0.322	0.096	0.1865	0.0170	0.0051
85	0.1698	0.9386	1.2175	2.519	0.219	0.301	0.1399	0.0122	0.0167
94	0.0429	0.4306	0.5554	0.922	0.131	0.046	0.1024	0.0145	0.0051
110	0.0431	0.7517	0.9931	1.018	0.154	-0.011	0.0636	0.0096	-0.0007
119	0.0075	0.3893	0.5323	0.449	0.078	0.048	0.0499	0.0087	0.0054
142	0.0039	0.8978	1.2488	0.689	0.187	0.020	0.0300	0.0081	0.0009
164	0.0000	0.7262	1.0997	0.343	0.256	0.098	0.0156	0.0116	0.0045
186	0.0000	0.6157	1.1002	0.140	0.385	0.030	0.0064	0.0175	0.0013

t, hr	Cumulative Mass Lost Through Membrane CH2Cl2 umol	CHCl3 umol	CCl4 umol	Effl Gas Flows sec	ml/min	L/hr
2	0.16	0.12	0.57	12.08	9.93	0.596
5	0.61	0.46	1.87	12.45	9.64	0.578
9	1.13	0.88	2.81	12.46	9.63	0.578
18	2.03	1.74	4.18	12.56	9.55	0.573
25	2.59	2.38	5.14	11.09	10.82	0.649
37	3.32	3.43	6.66	11.06	10.85	0.651
48	3.74	4.28	7.86	11.15	10.76	0.646
67	4.16	5.52	9.55	11.56	10.38	0.623
85	4.33	6.46	10.76	12.75	9.41	0.565
94	4.37	6.89	11.32	10.67	11.25	0.675
110	4.42	7.64	12.31	10.80	11.11	0.667
119	4.42	8.03	12.84	10.50	11.43	0.686
142	4.43	8.93	14.09	10.73	11.18	0.671
164	4.43	9.65	15.19	10.87	11.03	0.662
186	4.43	10.27	16.29	10.72	11.19	0.671

t, hr		CH2Cl2	CHCl3	CCl4
2		**********	**********	**********
5	Total Mass Degraded During Test, umol:	16.19	3.33	8.90
9				
18	Total Mass Lost Through Membrane, umol:	4.43	10.27	16.29
25				
37	Mass Remaining at End of Test, umol:	0.00	5.97	17.47
48				
67	Mass Initially Present, umol	20.62	19.57	42.66
85				
94				
110				
119				
142				
164				
186				

t, hr	dC/dt Due to Degradation CH2Cl2	CHCl3	CCl4	Liquid Phase C CH2Cl2	CHCl3	CCl4	C/(dC/dt) CH2Cl2	CHCl3	CCl4	Enzyme Kineti CH2Cl2 C	C/(dC/d
2	0.000	0.000	0.000	10.58	9.83	19.12				10.58	83.45
5	0.075	-0.005	0.658	10.07	9.59	13.39	133.9	-1824	20	10.07	80.04
9	0.039	0.025	0.281	9.61	9.22	10.96	246.6	364	39	9.61	76.96
18	0.017	0.021	0.070	8.96	8.53	9.10	532.9	401	129	8.96	72.62
25	0.055	0.022	0.086	8.31	8.04	8.65	151.2	361	101	8.31	68.27
37	0.109	0.023	0.009	6.70	7.30	8.16	61.5	316	903	6.70	57.51
48	0.122	0.014	0.004	5.21	6.74	7.72	42.7	466	1726	5.21	47.54
67	0.101	0.009	0.003	3.18	5.96	7.14	31.4	647	2594	3.18	33.97
85	0.076	0.007	0.009	1.81	5.38	6.60	23.8	814	726	1.81	24.81
94	0.056	0.008	0.003	1.32	5.10	6.41	23.7	646	2297	1.32	21.54
110	0.035	0.005	0.000	0.79	4.65	6.07	22.8	889	-17005	0.79	17.99
119	0.027	0.005	0.003	0.56	4.42	5.88	20.6	939	2016	0.56	16.45
142	0.016	0.004	0.000	0.22	3.89	5.43	13.5	882	11399	0.22	14.18
164	0.008	0.006	0.002	0.06	3.41	5.03	7.1	540	2072	0.06	13.11
186	0.003	0.010	0.001	0.00	2.92	4.61	0.0	307	6306	0.00	12.71

Line Equation for CH2Cl2 Hanes Linearization:

$y = 12.71 + 6.686x$

V_{max} = 0.150 umol/L-hr

K_m = 1.901 umol/L

t, hr
2
5
9
18
25
37
48
67
85
94
110
119
142
164
186

APPENDIX C

Electron Balance Spreadsheet

Electron Balance for Methylotrophic GPMS Biofilm

Time Line days	CH4 Consumed mmol/d	O2 Consumed mmol/d	N2 Produced mmol/d	CO2 Produced mmol/d	COD Consumed mg/cm^2-d
0.51	9.8	5.7	3.6	4.5	5.50
1.63	10.8	8.1	6.6	3.4	5.35
4.58	11.7	5.9	5.6	2.4	6.90
5.55	14.0	5.5	5.6	0.0	8.84
5.96	9.9	11.1	5.9	0.0	3.46
7.96	8.4	11.7	4.5	2.1	1.99
8.50	11.9	7.4	8.7	0.0	6.47
9.58	8.4	8.8	5.9	1.5	3.15
10.46	10.4	7.3	6.1	1.6	5.32
10.92	7.1	7.9	2.8	1.8	2.51
11.67	10.6	7.9	6.7	2.8	5.20
12.50	7.0	3.5	-0.1	1.9	4.09
19.76	14.5	7.1	2.0	2.5	8.64
20.00	15.9	18.7	5.9	2.2	8.36
20.35	11.9	7.1	-3.8	2.2	6.56
20.51	15.2	12.1	4.0	2.0	8.40
20.68	11.4	11.6	3.3	2.6	4.47
20.93	12.3	11.6	1.8	2.3	5.06
21.14	11.3	11.6	2.9	2.2	4.33
21.50	16.5	15.1	3.9	1.7	7.04
22.54	10.9	10.8	1.7	1.9	4.35
23.54	11.8	10.6	-0.4	2.6	5.14
28.54	10.2	9.1	-3.1	1.9	5.53
30.58	14.5	16.0	4.6	2.1	5.17
31.97	17.7	28.2	1.0	2.4	2.61
51.50	18.3	25.2	1.1	4.2	4.67
32.55	31.9	43.9	10.5	4.4	7.83
33.01	22.7	34.0	10.9	4.8	9.08
33.80	16.9	9.6	-7.6	3.7	9.54
34.52	19.9	26.8	2.7	3.7	5.39
34.93	19.1	20.7	2.0	2.7	6.90
35.56	12.8	-2.1	-15.3	3.8	3.77
35.97	12.3	15.1	0.8	3.9	3.76
36.93	12.6	15.6	0.3	4.3	4.16
37.81	13.4	13.6	0.4	4.2	5.21
38.76	13.1	14.5	0.4	4.2	4.67

Based on Stoichiometry of CH4 Oxidation
Using O2 and NO3- as Electron Acceptors

CH4 Ox'd by O2 mmol/d	CH4 Ox'd by NO3 mmol/d	Total CH4 Ox'd mmol/d	CH4 e- Donated e-mmol/d	O2 e- Accepted e-mmol/d	N2 e- Accepted e-mmol/d	Total e- Accepted e-mmol/d
5.0	4.8	9.8	49.1	24.6	38.6	63.2
7.0	3.8	10.8	54.0	34.9	70.8	105.7
5.1	6.6	11.7	58.8	25.4	60.1	85.5
4.8	9.2	14.0	70.5	23.7	60.1	83.8
9.7	0.2	9.9	49.1	47.8	63.3	111.1
10.2	-1.8	8.4	41.5	50.4	48.3	98.7
6.4	5.5	11.9	59.6	31.9	93.4	125.2
7.7	0.7	8.4	41.7	37.9	63.3	101.2
6.4	4.0	10.4	52.0	31.5	65.5	96.9
6.9	0.2	7.1	35.2	34.0	30.0	64.1
6.9	3.7	10.6	53.0	34.0	71.9	105.9
3.0	4.0	7.0	35.2	15.1	-1.1	14.0
6.2	8.3	14.5	72.8	30.6	21.5	52.1
16.3	-0.4	15.9	78.8	80.6	63.3	143.9
6.2	5.7	11.9	59.7	30.6	-40.8	-10.2
10.5	4.7	15.2	75.9	52.2	42.9	95.1
10.1	1.3	11.4	56.7	50.0	35.4	85.4
10.1	2.2	12.3	61.3	50.0	19.3	69.3
10.1	1.2	11.3	56.2	50.0	31.1	81.1
13.1	3.4	16.5	82.2	65.1	41.8	106.9
9.4	1.5	10.9	54.2	46.5	18.2	64.8
9.2	2.6	11.8	58.8	45.7	-4.3	41.4
7.9	2.3	10.2	50.8	39.2	-33.3	6.0
13.9	0.6	14.5	72.0	69.0	49.4	118.3
24.5	-6.8	17.7	87.0	121.5	10.7	132.3
21.9	-3.6	18.3	90.4	108.6	11.8	120.4
38.2	-6.3	31.9	157.5	189.2	112.7	301.9
29.6	-6.9	22.7	111.8	146.5	117.0	263.5
8.4	8.5	16.9	84.8	41.4	-81.5	-40.2
23.3	-3.4	19.9	98.3	115.5	29.0	144.5
18.0	1.1	19.1	94.9	89.2	21.5	110.7
-1.8	14.6	12.8	65.1	-9.1	-164.2	-173.2
13.1	-0.8	12.3	60.9	65.1	8.6	73.7
13.6	-1.0	12.6	62.4	67.2	3.2	70.5
11.8	1.6	13.4	66.6	58.6	4.3	62.9
12.6	0.5	13.1	65.0	62.5	4.3	66.8

APPENDIX D

Energetics Analyses

Energetics Analyses for Aerobic Methane Degradation

Case 1. Assume CH_4 is the electron donor and O_2 is the electron acceptor. NO_3^- is the nitrogen source.

Energy Reaction

ED: CH_4
EA: O_2

RD: $1/8\ CH_4 + 1/4\ H_2O \longrightarrow 1/8\ CO_2 + H^+ + e^-$ G = -5.763 kcal

RA: $e^- + H^+ + 1/4\ O_2 \longrightarrow 1/2\ H_2O$ G = -18.675 kcal

Overall Energy Reaction:

$1/8\ CH_4 + 1/4\ O_2 \longrightarrow 1/8\ CO_2 + 1/4\ O_2$

G_r = RD + RA = -5.763 - 18.675 = -24.44 kcal/e-mol

Pyruvate Formation

ED: CH_4
EA: CO_2

RD: $1/8\ CH_4 + 1/4\ H_2O \longrightarrow 1/8\ CO_2 + H^+ + e^-$ G = -5.763 kcal

RA: $1/5\ CO_2 + 1/10\ HCO_3^- + H^+ + e^- \longrightarrow 1/10\ CH_3COCOO^- + 2/5\ H_2O$

G = 8.965 kcal

G_p = RD + RA = 3.20 kcal/e-mol

G_c = 7.5 kcal/e-mol

G_n = 4.2 kcal/e-mol

$$A = -\frac{G_p/k^m + G_c + G_n/k}{k\,G_r} = -\frac{3.20/0.6 + 7.5 + 4.2/0.6}{0.6(-24.44)} = 1.352$$

Synthesis Reaction

ED: CH_4
EA: CO_2

RD: $1/8\ CH_4 + 1/4\ H_2O \longrightarrow 1/8\ CO_2 + H^+ + e^-$

RA: $1/28\ NO_3^- + 5/28\ CO_2 + 29/28\ H^+ + e^- \rightarrow$

$$1/28\ C_5H_7O_2N + 11/28\ H_2O$$

Overall Synthesis Reaction:

$$1/8\ CH_4 + 1/28\ NO_3^- + 0.054\ CO_2 + 1/28\ H^+ \rightarrow 1/28\ C_5H_7O_2N + 1/7\ H_2O$$

Overall Reaction = Synthesis Reaction + A(Energy Reaction)

Overall Reaction Based on Energetics:

$$0.294\ CH_4 + 0.036\ NO_3^- + 0.036\ H^+ + 0.338\ O_2 \rightarrow$$

$$0.036\ C_5H_7O_2N + 0.115\ CO_2 + 0.481\ H_2O$$

Case 2. Assume CH_4 is the electron donor and NO_3^- is the electron acceptor. NO_3^- is also the nitrogen source.

Energy Reaction

ED: CH_4
EA: NO_3^-

RD: $1/8\ CH_4 + 1/4\ H_2O \rightarrow 1/8\ CO_2 + H^+ + e^-$ $\quad G = -5.763$ kcal

RA: $1/5\ NO_3^- + 6/5\ H^+ + e^- \rightarrow 1/10\ N_2 + 3/5\ H_2O$ $\quad G = -17.128$ kcal

Overall Energy Reaction:

$$1/8\ CH_4 + 1/5\ NO_3^- + 1/5\ H^+ \rightarrow 1/8\ CO_2 + 1/10\ N_2 + 3/5\ H_2O$$

$G_r = RD + RA = -22.891$ kcal/e-mol

Pyruvate Formation

ED: CH_4
EA: CO_2

Therefore, same as Case 1.

$G_p = 3.20$ kcal/e-mol

$G_c = 7.5$ kcal/e-mol

$G_n = 4.2$ kcal/e-mol

$A = 1.444$

Synthesis Reaction

ED: CH_4
EA: CO_2

Therefore, same as Case 1.

Overall Synthesis Reaction:

$$1/8\ CH_4 + 1/28\ NO_3^- + 0.054\ CO_2 + 1/28\ H^+ \rightarrow 1/28\ C_5H_7O_2N + 11/28\ H_2O$$

Overall Reaction = Synthesis Reaction + A(Energy Reaction)

Overall Reaction Based on Energetics:

$$0.306\ CH_4 + 0.324\ NO_3^- + 0.324\ H^+ \longrightarrow$$
$$0.036\ C_5H_7O_2N + 0.126\ CO_2 + 0.144\ N_2 + 0.648\ H_2O$$

Electron Balance - Case 1:

Molecule	Element	Number	Charge	Total Charge
Reactants:				
CH_4	C	1(0.294)	-4	-1.176
	H	4(0.294)	+1	+1.176
NO_3^-	N	1(0.036)	+5	+0.180
	O	3(0.036)	-2	-0.216
O_2	O	2(0.338)	0	0
H^+	H	1(0.036)	+1	+0.036
				0
Products:				
$C_5H_7O_2N$	C	5(0.036)	-1	-0.180
	H	7(0.036)	+1	+0.252
	O	2(0.036)	-2	-0.144
	N	1(0.036)	+2	+0.072
CO_2	C	1(0.115)	+4	+0.460
	O	2(0.115)	-2	-0.460
H_2O	H	2(0.481)	+1	+0.962
	O	1(0.481)	-2	-0.962

Balance:

		Change
C:	-1.176 --> -0.180 + 0.460	+1.456
H:	+1.176 + 0.036 --> +0.252 + 0.962	+0.002
N:	+0.180 --> +0.072	-0.108
O:	-0.216 + 0 --> -0.144 - 0.460 - 0.962	-1.350

Therefore,

4.96 e-mol donated/mol CH_4 oxidized

4.31 e-mol accepted/mol O_2 reduced

0.870 mol CH_4 oxidized/mol O_2 reduced

By a similar development, with nitrate as the primary electron acceptor (Case 2),

5.07 e-mol donated/mol CH_4 oxidized

10.73 e-mol accepted by NO_3^-/mol N_2 produced

2.12 mol CH_4 oxidized/mol N_2 produced